



Protein-membrane interaction: insights from advanced microscopy

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Oral Presentations
– Plenary Lectures –

O-1

The termination of translation in bacteria and eukaryotes

V. Ramakrishnan

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Protein synthesis is terminated when the end of the coding sequence is reached when one of three stop codons enters the A site of the ribosome. This results in the binding of a protein “class I” release factor to the ribosome and cleavage of the newly made protein from the P-site tRNA. Surprisingly for such a universal function, these factors are completely unrelated in bacteria and in eukaryotes. Almost a decade ago, we initiated studies on bacterial translational termination by crystallography. Recently we used some of the remarkable advances in electron microscopy to determine the structures of eukaryotic termination complexes. A comparison of the bacterial and eukaryotic cases shows how termination is carried out in very different ways in the two domains of life. We also show how a quality control factor can recruit release factor in the complete absence of a codon to stimulate peptide release.

O-3

Single cell genomics: stochasticity meets precision in biology and medicine

S. Xie^{1,2}

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DNA exists as single molecules in individual cells. Consequently, gene expression is stochastic. High precision single cell transcriptome measurements reveal intrinsic correlations among different genes, which is masked within the stochastic noise. The fact that there are 46 different individual DNA molecules (chromosomes) in a human cell dictates that genomic variations occur stochastically and cannot be synchronized among individual cells. Probing such genomic variations requires single cell and single molecule measurements, which have only recently been made possible, opening opportunities to investigate and to diagnose cancer, and to avoid genetic disorders in newborns.

O-2

Towards a mechanistic understanding of ribosomal function

L. V. Bock, M. Kolar, A. C. Vaiana, H. Grubmüller

Max Planck Institute for Biophysical Chemistry, Germany

During protein synthesis, tRNA molecules move from the ribosome’s aminoacyl to peptidyl to exit sites, with the two ribosomal subunits remaining associated through a complex network of intersubunit (ISU) bridges, despite rapid large-scale ISU rotation. Using molecular dynamics simulations, we investigated conformational motions during spontaneous translocation, as well as the underlying energetics and kinetics. We asked how binding affinity between the two subunits is controlled and maintained at a quite constant level despite large-scale motions and highly dynamic changes. Indeed, ISU rotations exhibit remarkably fast intrinsic sub-microsecond dynamics, which requires a fine-tuned flat free energy landscape, as any larger barrier would slow down the conformational motions. The total contribution of the tRNAs to the ISU binding enthalpy is almost constant, despite their changing positions in the ribosome during translocation. These mechanisms keep the ISU interaction strong and steady during rotation, thereby preventing dissociation and enabling rapid rotation. We further describe a new combined allosteric mechanism for erythromycin-induced translational stalling of the antibiotics sensor peptide ErmB as well as nascent peptide dynamics in the ribosomal exit tunnel.

O-4

Towards mass spectrometry of single molecules with light

P. Kukura

Physical and Theoretical Chemistry Laboratory, University of Oxford, UK

Mesoscopic structure and dynamics are central to a variety of key processes in nature, such as crystallisation, force generation or metastasis. An impressive number of techniques have been developed over the past decades aimed at understanding the underlying mechanisms, leading to remarkable levels of knowledge with respect to both molecular and in some cases atomic structure as well as the overall kinetics of (dis)assembly of the relevant macromolecular complexes and structures. A distinct challenge, however, remains regarding our ability to directly visualise and thereby quantify these interactions and dynamics at the fundamental, single molecule level. I will show how ultrasensitive optical microscopy based on light scattering alone, in the form of interferometric scattering microscopy (iSCAT), can be used to study mesoscopic dynamics with single molecule sensitivity, specificity and resolution. I will illustrate these capabilities with studies of phase separation, interfacial dynamics and biological filaments and present recent results that demonstrate the capability of iSCAT to operate as a single molecule mass spectrometer in solution, which could be transformative for our ability to study protein-protein and protein-drug interactions.

Oral Presentations

– Plenary Lectures –

O-5

The challenges and opportunities of understanding protein folding and misfolding in health and disease

S. E. Radford

The University of Leeds, UK

The last decade has seen astounding increases in our knowledge of how proteins fold, both *in vitro* and in living cells. Based on experiments with ever-increasing levels of sophistication and power, we can now monitor folding transitions on μ sec-msec timescales in all atom detail, watch proteins folding as they first emerge from the ribosome, and witness the conformational fluctuations of individual protein molecules as they search for their native conformations on rugged energy landscapes. These approaches are now also being applied to membrane proteins, revealing details of the pathways and chaperones involved in folding into a lipid bilayer in different cellular compartments. It is also now clear that proteins can misfold, sometimes frequently during folding. If left unchecked by molecular chaperones, misfolding can result in disastrous consequences for the cell. Understanding the fundamental mechanisms of protein folding, how misfolded proteins aggregate and how these species are recognised, are also rapidly moving and exciting fields.

In this lecture I will summarise some of the exciting advances in our understanding of protein folding *in vitro* and in the cell, and discuss our current understandings of the molecular mechanisms of aggregation drawn from biophysical approaches.

O-7

A novel cross-talk between membrane lipids and the innate system is mediated by toll-like receptors

J.-M. Ruysschaert

Free University of Brussels, Belgium

Toll-like receptors (TLR) are innate system proteins that serve as sensors of microbial molecules and contribute to first defense against bacterial and viral infection. In response to TLRs engagement a signal transmitted to the cell that brings the immune system in a “state of emergency”, ready to react to a microbial invasion.

It was recently reported (1) that non bacterial ligands that bear no structural similarity to established TLR ligands recognise TLRs and activate innate immunity cascades. This new concept suggest that a diversity of non structurally related molecules activate TLR receptors and are not as inert and safe as we thought. We will illustrate this view with a few examples. These unexpected inflammatory reactions can be desired (for vaccine development), unwanted (for delivery applications) or involved in the induction of non-infectious diseases like amyloidosis. An improved knowledge of the relationship between the lipid properties (nature of the hydrophilic moieties, hydrocarbon tails, mode of organisation) and the activation of the innate pathways via TLRs opens the way to the design of new molecules tailored for specific applications in human cells (gene transport, adjuvant), plant cells and to therapeutic perspectives largely unintended until now.

1) Lonez C, Vandenbranden M, Ruysschaert JM.-Adv Drug Deliv Rev. 2012;64,1749-58

O-6

Resource allocation theory of bacterial physiology

T. Hwa

University of California San Diego, USA

The ultimate goal of systems biology is to attain a quantitative, predictive understanding of the behavior of organisms as a whole (i.e., physiology) from the molecular parts. A major obstacle to this endeavor is the enormous number of mostly inaccessible parameters needed to characterize even the simplest organisms. Here, I will describe how quantitatively accurate predictions on bacterial growth and metabolism can be attained using a top-down coarse-graining approach based on a simple principle of proteomic resource allocation. This principle is established and validated by extensive experiments for exponentially growing bacteria. A kinetic extension of this principle leads to a single differential equation which is able to predict quantitatively the time course of flux and gene expression during nutrient upshift, downshift, and upon antibiotic inhibition, all without the need of kinetic parameters. These findings reveal an underlying strategy of growth control that is surprisingly simple, rigid, and generic.

O-8

Biophysics with minimal biological systems

P. Schwillie

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Biophysical methodology has been instrumental in vastly increasing our knowledge about biological systems in the last decades. Today, we can zoom much deeper into cells and molecules than ever, and quantitatively digest huge amounts of data that have long been impossible to think of. On the other hand, the understanding of biology from first principles, which is usually the primary goal of physics, has moved the more out of reach the more we learned about the incredible complexity of living systems. In my talk I want to advocate a new approach towards understanding biology, which is concerned with designing biological systems from the bottom-up, which nevertheless resemble essential features of life. I will particularly highlight new advances towards reconstituting a minimal version of bacterial cell division.

Oral Presentations**– Plenary Lectures –****O-9****Seven transmembrane receptors**R. J. Lefkowitz^{1,2}¹Howard Hughes Medical Institute, USA; ²Duke University Medical Center, USA

Seven transmembrane receptors (7TMRs), also known as G protein coupled receptors (GPCRs) represent by far the largest, most versatile, and most ubiquitous of the several families of plasma membrane receptors. They regulate virtually all known physiological processes in humans. As recently as 40–50 years ago, the very existence of cellular receptors for drugs and hormones was highly controversial, and there was essentially no direct means of studying these putative molecules. Today, the family of GPCRs is known to number approximately 1,000, and crystal structures have recently been solved for more than two dozen members of the family and even of a receptor-G protein complex. I will briefly review how the field has evolved over the past 50 years, hanging some of the story on my own research. Then I will discuss recent developments in the field, which are changing in fundamental ways our concepts of how the receptors function and are regulated. These include the duality of signaling through G-proteins and β -arrestins; the development of “biased ligands”; and the possibility of leveraging this new mechanistic and molecular information to develop new classes of therapeutic agents. Finally, I will discuss recent biophysical and structural studies of receptor- β -arrestin interactions.

Oral Presentations**– 1. Multiscale biophysics of membranes –****O-10****Mechanisms of Membrane Curvature Generation**

T. Baumgart
University of Pennsylvania, USA

Membrane curvature has developed into a forefront of membrane biophysics. Numerous proteins involved in membrane curvature sensing and membrane curvature generation have recently been discovered, including proteins containing the crescent-shaped BAR domain as membrane binding and shaping module. Accordingly, the structure of these proteins and their multimeric complexes is increasingly well-understood.

Substantially less understood, however, are the detailed mechanisms of how these proteins interact with membranes in a curvature-dependent manner. New experimental approaches need to be combined with established techniques to be able to fill in these missing details. Here we use model membrane systems in combination with a variety of biophysical techniques to characterize mechanistic aspects of BAR domain protein function. This includes a characterization of membrane curvature sensing and membrane generation. We present a new approach to investigate membrane curvature transitions, and introduce membrane shape stability diagrams as a powerful tool to enhance the mechanistic understanding of membrane trafficking phenomena, including endocytosis, with molecular detail.

O-12**Physical model of cellular organelles biogenesis and transport**

P. Sens
Institut Curie, Paris, France

Membrane-bound organelles perform essential cellular functions such as the sorting and biochemical maturation of cellular components. They are strongly out-of-equilibrium structures and display large stochastic fluctuations of composition and shape resulting from inter-organelle exchange and enzymatic reactions. Understanding how the molecular mechanisms controlling these processes are orchestrated is an outstanding problem of great interest for cell biologist, but also for physicists.

In this talk, I will first discuss a quantitative model of protein transport through the Golgi apparatus and compare it with experimental data to determine the contribution to intra-Golgi transport of the two - often opposed - dynamical model; *cisternal maturation* and *inter-cisternal exchange*.

I will then discuss a conceptual model of organelle biogenesis and maintenance that includes vesicular exchange between organelles and the biochemical maturation of an organelle's content. I will show how the non-equilibrium steady-state of an organelle or a network of organelles may be varied in a controlled manner by modifying a few coarse-grained parameters (essentially, the budding, fusion and maturation rates) and discuss the relevance of these results for the structure of the Golgi apparatus.

O-11**Structure on the nanoscopic scale of complex biomembrane mimetics**

G. Pabst^{1,2}

¹University of Graz, Institute of Molecular Biosciences, NAWI Graz, Austria; ²BioTechMed-Graz, Austria

Lipid-only models serve as valuable platforms for studying the functional role of membrane lipids in biological cells under chemically and experimentally well-defined conditions. Of recent, we have particularly focused on fully hydrated lipid vesicles with either lateral or transbilayer inhomogeneities applying a combination of X-ray/neutron scattering experiments, solution NMR and other complimentary techniques. This allowed us to address specific questions related to structural details of liquid-ordered (Lo) and liquid-disordered (Ld) domains, interleaflet coupling or passive lipid flip-flop. Specifically we found (i) a strong correlation of the Lo/Ld mismatch between lipid packing density and domain thickness with domain size, (ii) significant coupling between gel-fluid membrane leaflets and (iii) slow lipid flip-flop far away from the melting transition in the gel and fluid phases, with a significant boost in the transition regime. Results will be discussed with respect to potential effects on transmembrane proteins.

O-13 (P-1)**Developing ESCRT-III as a toolkit for bottom-up construction of eukaryote-like artificial cells**

A. Booth¹, C. Marklew², B. Ciani², P. A. Beales¹

¹University of Leeds, UK; ²University of Sheffield, UK

The Endosomal Complex Required for Transport (ESCRT) is a ubiquitous class of proteins involved in most membrane remodeling processes in eukaryotic cells. Previous studies have demonstrated that ESCRTs can be reconstituted in artificial lipid vesicle systems, notably giant unilamellar vesicles (GUVs), where they are able to induce the formation of intraluminal vesicles (ILVs), encapsulating material from the extravesicular solution.^[1]

Our aim is to develop the ESCRT proteins as a toolkit to generate GUV-derived structures, with multiple compartments containing different chemical environments, enzymes or molecular probes. The resulting cell-like structures may find applications in synthetic biology, drug delivery, diagnostics and the development of nano-reactor technology.^[2]

Using confocal microscopy and flow cytometry, we have gained insights into how protein stoichiometry and membrane mechanics influence the size and number of the ILV compartments obtained. We will further develop the ESCRT toolkit using the ATP-ase Vps4 to induce multiple encapsulation events and introduce rationally designed ESCRT chimera proteins to further simplify the system.

[1] T. Wollert, J. Lippincroft-Schwartz, J. H. Hurley, Nature, 2009

[2] P. A. Beales, B. Ciani, Phys. Chem. Chem. Phys., 2015

Oral Presentations**– 1. Multiscale biophysics of membranes –****O-14 (P-2)****Anomalous diffusion in artificial lipid bilayers**H. L. Coker^{1,2}, M. R. Cheetham², M. I. Wallace²¹University of Oxford, UK; ²King's College London, UK

In contrast to artificial bilayers, diffusion in cells is slower and non-ergodic. The physical origins of anomalous diffusion are poorly understood; cytoskeletal-membrane interactions and nanoscopic roughness have been suggested as mechanisms. We use a bottom-up approach to create mimics of anomalous behaviour to better understand the factors that control diffusion. Using a combination of single-molecule fluorescence and interferometric scattering microscopy we study diffusive behaviours at microsecond time resolution over several seconds.

In a supported bilayer model with PEG-modified lipids, we can control free diffusion: as the concentration of PEG-lipids increases, bilayer contiguity is disrupted. We observe a dramatic and controllable switch in anomalous diffusion as we cross the percolation threshold that is dependent on the length of PEG modification. Our results are well described using a fractal model of tracer diffusion.

To address the role of topography, we generate bilayers on glass substrates with square-wave 'nanowall' patterning. Although fluorescence tracking reveals anisotropic diffusion, this is not anomalous over any timescale. This reveals that topological confinement, as opposed to topography, is required for anomalous diffusion in lipid bilayers.

O-15 (P-3)**Cholesterol and polyunsaturated lipids working in concert to modulate G protein-coupled receptors**M. Javanainen^{1,2}, M. Manna¹, M. Vähäheikkilä¹, M. Niemelä¹, J. Tynkkynen¹, R. Gùixa-González³, H. Martinez-Seara^{1,4}, G. Enkavi^{1,2}, W. Kulig², D. J. Müller⁵, T. Rog^{1,2}, I. Vattulainen^{1,2}¹Lab. of Physics, Tampere Univ. of Technology, Finland;²Dept. of Physics, Univ. of Helsinki, Finland; ³Inst. of Medical Physics & Biophysics, Charité Universitätsmedizin Berlin, Germany; ⁴Inst. of Organic Chemistry & Biochemistry, Prague, Czech Republic; ⁵Dept. of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland

G protein-coupled receptors (GPCRs) are signaling machines that constitute the largest family of integral membrane proteins in human genome. Given that many GPCRs contain binding sites for specific lipids, there is reason to assume that lipids play a key role in the stability, dynamics, and activation of various GPCRs. However, the critical barrier to progress in the field has been the lack of data showing in atomistic detail how *exactly* lipids modulate GPCRs. Here we overcome this challenge through extensive self-assembly and equilibrium simulations of both atomistic and coarse-grained models of multi-component lipid membranes with GPCRs. Through considerations of many proteins (e.g., the human β_2 -adrenergic receptor, the adenosine A2A receptor, etc.), we discuss how GPCRs reorganize their membrane environment to create domains that promote their function. In these domains, cholesterol is one of the central lipids, and we discuss how it can modulate GPCR stability, dynamics, and conformation in an allosteric fashion. Meanwhile, lipids with polyunsaturated fatty acids turn out to be decisive in modulating GPCR partitioning in different membrane environments. Overall, lipids modulate GPCRs in a largely concerted manner that becomes evident in multi-component membrane regions.

Oral Presentations**– 2. Molecular machinery –****O-16****Shape remodeling of active cytoskeletal vesicles**

A. R. Bausch

Technical University Munich, Germany

Living cells rely on the self-organization mechanisms of cytoskeleton to adapt to their requirements. Most processes such as cell division, or cellular motility rely on the controlled self-assembly of well defined active cytoskeletal structures interacting with membrane, which still allow a dynamic reorganization. One important and promising strategy to identify the underlying governing principles is to quantify the physical process in model systems mimicking the functional units of living cell. Here I'll present *in vitro* minimal model systems consisting of active microtubule or actin filament systems into lipid vesicles. I will discuss how a balance of local force exertion and tension generation results in shape transformations, blebbing, invagination or tethering of lipid membranes.

O-18**Navigating the cytoskeleton: using light to dissect and direct intracellular transport**

L. C. Kapitein

Utrecht University, Netherlands

Proper positioning of organelles by cytoskeleton-based motor proteins underlies cellular events such as signaling, polarization, and growth. For example, the selective transport of different cargoes into axons and dendrites underlies the polarized organization of the neuron, whereas the regulated intra-dendritic transport of receptor-carrying endosomes is important for synaptic maintenance and modulation. To explore how different motor proteins contribute to transport and to study the site-specific roles of different organelles, we have recently established optical control of intracellular transport by using light-sensitive heterodimerization to recruit specific cytoskeletal motor proteins (kinesin, dynein or myosin) to selected cargoes. In addition, to better understand how the specialized organization of the neuronal cytoskeleton contributes to intracellular transport, we have developed novel approaches for light-based nanoscopy. In this lecture, I will demonstrate how these technologies advance our understanding of neuronal transport.

O-17**Engineering controllable biomolecular motors**

Z. Bryant

Stanford University, USA

Engineering biomolecular motors can provide direct tests of structure-function relationships, new tools for controlling cellular processes, and customized components for harnessing molecular transport in artificial systems. Our laboratory has previously introduced a series of modified cytoskeletal motors that reversibly change gears — speed up, slow down, or switch directions — when exposed to external signals such as metal ions or blue light. We have recently expanded the functional range of optically controlled myosins, creating next-generation designs with optimized velocities and deep modulation depths. We have also designed and tested myosin motors that incorporate RNA lever arms, forming hybrid assemblies in which conformational changes in the protein motor domain are amplified and redirected by nucleic acid structures. Using *in vitro* assays of propelled actin filaments and single-molecule tracking of processive complexes, we have confirmed the operation of RNA-myosin motors designed to reversibly change direction in response to oligonucleotide signals that drive strand-displacement reactions. Together, an extended set of optogenetic motors and RNA-protein hybrid motors will provide a diverse toolkit for programmable control of nanoscale transport and force generation.

O-19 (P-57)**Structural dynamics of the 70S ribosome during translocation monitored by single-molecule FRET**

S. Adio, H. Sharma, T. Senyushkina, F. Peske, M. V. Rodnina

MPI biophysical Chemistry, Göttingen, Germany

Ribosome dynamics play an important role in translation. The rotation of the ribosomal subunits relative to one another is essential for tRNA-mRNA translocation. We monitor subunit rotation relative to peptide bond formation and translocation using ensemble kinetics and single-molecule FRET. We find that large-scale movements of the ribosome are intrinsically rapid and gated by its ligands such as EF-G and tRNA.

The coupled translocation of tRNA and mRNA through the ribosome includes step-wise movements of the tRNAs. Structural work has visualized intermediates of translocation induced by EF-G with tRNAs trapped in chimeric states with respect to 30S and 50S ribosomal subunits. The functional role of the chimeric states is not known. We follow the formation of translocation intermediates by single-molecule FRET. Using EF-G mutants, a non-hydrolysable GTP analogue, and the antibiotic fusidic acid, we interfere with either translocation or EF-G release from the ribosome and identify several rapidly interconverting chimeric tRNA states on the reaction pathway. Our data illustrates that the engagement of EF-G alters the energetics of translocation towards a flat energy landscape, thereby promoting forward tRNA movement.

Oral Presentations**– 2. Molecular machinery –****O-20 (P-58)****Stepping motion and chemo-mechanical coupling of chitinase resolved by single-molecule analysis**

R. Iino, A. Nakamura

National Institutes of Natural Sciences, Japan

Serratia marcescens chitinase A (SmChiA) is a monomeric linear molecular motor moving on and hydrolyzing crystalline chitin processively. Here we directly resolved steps and pauses in the motion of SmChiA with high-resolution single-molecule imaging analysis. By using total internal reflection dark-field microscopy and 40-nm gold nanoparticle as a low-load probe, movement of SmChiA was observed at 1,000–2,000 frames/s with 0.3 nm localization precision. The step sizes were 1.1 nm and -1.3 nm for forward and backward steps, respectively, consistent with the length of the product, chitobiose (~1 nm). The ratio of forward to backward steps was 2.9, corresponding to the energy difference of 1.1 $k_B T$. Frequent backward steps and low energy difference indicate that SmChiA operate as the Brownian ratchet. Furthermore, detailed analysis of the distribution of pause duration revealed that the rate-limiting step of chemo-mechanical coupling of SmChiA is the decrystallization of single polymer chain from the crystalline chitin, not bond cleavage and product release. These results give us important insights to engineer non-natural chitinases which show better performances than the natural ones.

O-21 (P-59)**Single-molecule dissection of cytoplasmic dynein force sensing**L. Rao¹, F. Berger², M. P. Nicholas¹, A. Gennerich¹¹Albert Einstein College of Medicine, USA; ²Rockefeller University, USA

Cytoskeletal motor protein motility requires coordination of ATPase and filament-binding cycles. Mechanical tension, which arises from both external and intramolecular forces, regulates motor stepping. In cytoplasmic dynein – a unique AAA+ ATPase with six linked AAA+ domains (AAA1–6), a coiled-coil stalk that connects the AAA+ ring and microtubule (MT)-binding domains, and a dimerizing tail domain – applied tension affects microtubule (MT)-binding strength anisotropically, with backward tension inducing stronger binding. Using optical tweezers, mutagenesis, and chemical cross-linking, we elucidate the underlying molecular mechanisms of this behavior, showing that either preventing relative motion of the dynein stalk helices or deleting the coiled coil ‘strut’ that emerges from AAA5 and contacts the stalk eliminate tension-based regulation of MT-binding. In addition, linker docking/undocking to and from AAA5 controls tension-induced stalk reconfiguration. Thus, tension alters dynein’s MT-binding strength by inducing sliding of the stalk helices, mediated by the strut and linker.

Oral Presentations

– 3. Quantitative approaches to gene regulation –

O-22

Nutrient dependent levels of SBF and MBF are key factors controlling Start in budding yeast

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How organisms coordinate cell growth and division to maintain a homeostatic cell size remains unresolved. The commitment to division in budding yeast (Start) is associated with transcription of the G1/S regulon controlled by the activators, MBF and SBF, and the SBF repressor, Whi5. We have used a particle counting technique, scanning Number and Brightness (sN&B) to measure the absolute concentrations and oligomeric state of these factors in single live G1 yeast cells grown on rich and poor media. On glucose, they exhibited low nuclear concentrations (70–160nM) and were dimers. The activator, Swi6, was limiting for its DNA-binding partners, Swi4 and Mbp1. MBF and SBF copy numbers were insufficient to saturate their target DNA sites in small cells, but increased with cell size. Unlike wild-type, the *mbp1* deletion mutant was only slightly smaller on poor compared to rich medium, and sN&B demonstrated upregulation of MBF components (+50%) on glycerol, reflecting an enhanced role for MBF in the small cell size phenotype on poor nutrients. Mathematical modeling constrained by the sN&B results revealed the [Swi4]/[Whi5] ratio and the fraction of SBF/MBF-bound DNA sites as previously unknown key determinants of Start.

O-24 (P-82)

Nucleosome mobility and the regulation of gene expression: insights from single-molecule studies

S. Rudnizky, O. Malik, A. Bavly, L. Pnueli, P. Melamed, A. Kaplan

Technion - Israel Institute of Technology, Israel

Nucleosomes at the promoters of genes regulate the accessibility of the transcription machinery to DNA, and function as a basic layer in the complex regulation of gene expression. Our understanding of the role of the nucleosome's spontaneous, thermally driven position changes in modulating expression is lacking. This is the result of the paucity of experimental data on these dynamics, at high-resolution, and for DNA sequences that belong to real, transcribed genes. We have developed an assay that uses partial, reversible unzipping of nucleosomes with optical tweezers to repeatedly probe a nucleosome's position over time. Using the nucleosomes at the promoters of two model genes, *Cga* and *Lhb*, we show that the mobility of nucleosomes is modulated by the sequence of DNA and by the use of alternative histone variants, and describe how the mobility can affect transcription, at the initiation and elongation phases.

O-23

Reconstructing human developmental networks using single-cell RNA-seq

B. Treutlein^{1–3}

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Recent advances in the field of stem cell biology have made it possible to model human developmental processes in vitro. We combine stem cell based in vitro systems with single-cell transcriptomics analysis to reconstruct human organ development. In my talk I will present our efforts on reconstructing developmental networks during human liver development.

We dissect a 3-D liver organoid system that is generated by reconstituting hepatic, stromal, and endothelial cell interactions occurring during liver bud (LB) development. We compare hepatocyte-like lineage progression from pluripotency in 2-D culture and 3-D LB organoids and find that hepatoblasts in the 3D microenvironment differentiate towards hepatocyte fate, but also express epithelial migration signatures of organ budding. Strikingly, hepatoblasts in 3D LB organoids are more similar to primary human fetal hepatocytes than hepatoblasts in 2D monoculture. We use network analysis to predict autocrine and paracrine signaling in LBs, and predict inter-lineage communication involved in LB vascularization and self-organization. Our molecular dissection of a human organoid system illuminates previously inaccessible aspects of human development.

O-25 (P-81)

Heat triggers specific mRNA localization to regulatory RNA-protein granules in budding yeast

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Eukaryotic cells form large RNA-protein granules in response to stress. Here we measure the RNA content of heat-shock granules or aggregates by sequencing RNA from distinct fractions of cell lysate. We find that most messenger RNA species (mRNAs) aggregate specifically in response to heat shock. This is consistent with our reported measurements of heat-aggregated proteins, which were enriched in RNA-binding proteins. However, many specific mRNAs are excluded, including many messages that are abundant prior to heat shock, and also newly transcribed heat-shock protein mRNAs. Proteins are synthesized from only a subset of non-aggregating RNAs. No mRNAs remain aggregated during recovery at normal growth temperature, consistent with observed reversible aggregation of protein components of heat shock granules. RNA-binding proteins and their measured mRNA binding partners may aggregate together, or independently, suggesting stress-triggered unbinding. Our results are consistent with heat-shock granules being sites of temporary translational repression for specific mRNAs, acting in parallel with transcription, translation, and degradation of RNA to remodel cells in response to stress.

Oral Presentations**– 3. Quantitative approaches to gene regulation –****O-26 (P-349)****Beyond sequence: implications of DNA structure and dynamics in genome function**

A. Noy

Department of Physics, Biological Physical Sciences Institute, University of York, UK

DNA looping and folding are essential features for the function of genomes, highlighting the importance of studying the physical properties of DNA and its interacting molecules.

I have explored the length-dependence of DNA elasticity to understand the origin of the contradictory experimental measurements obtained at the micro and meso-scale. While classical single-molecule force-extension techniques described DNA as a rigid polymer, novel experimental methods have suggested DNA is more flexible at smaller scales. I found that DNA elasticity varies on the molecule's length in surprising ways, thus unravelling the experimental contradiction [1].

Moreover, I have been interested to describe how DNA deformations can be transmitted through the DNA fibre itself or across DNA loops in a 1D or 3D mechanism of communication, respectively. In the former, we detected that the probability for a DNA side to melt not only depends of local sequence but also of the whole topological domain [2]. In the latter, I found the enhanced capacity of supercoiled DNA plectonemes to promote protein-DNA interactions in relation to relaxed loops [3].

1. Noy et al, *Phys Rev Lett*, (2012), 109, 228101.

2. Sutthibutpong et al. *Nuc Acids Res*, (2016). 44, 9121.

3. Noy et al. *Biophys J*, (2017), 112, 523.

Oral Presentations

– 4. Synthetic biology –

O-27

Programmable on-chip DNA compartments as ‘artificial cells’

R. Bar-Ziv

Weizmann Institute of Science, Rehovot, Israel

The assembly of artificial cells capable of executing DNA programs has been an important goal for basic research and technology. We assemble 2D DNA compartments fabricated in silicon as ‘artificial cells’ capable of metabolism, programmable protein synthesis, and communication. We programmed gene expression cycles in separate compartments, as well as protein synthesis fronts propagating in a coupled 1D system of compartments. Gene expression in the DNA compartments reveals a rich, dynamic system that is controlled by geometry. The organization of matter in the compartment suggests conditions for controlled assembly of biological machines. This puts forth a man-made biological system with programmable information processing from the gene to a ‘cell’, and up to the ‘multicellular’ scale.

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O-29 (P-86)

Artificial cell reactor array technology

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We developed two classes of array devices displaying over one million of femto-liter reactors (FR); water-in-oil droplet type and lipid bilayer-sealed type. Both of them have size in a few microns and volume of femtoliters. We have demonstrated digital bioassays with droplet FR; digital ELISA, and digital virus counting. Droplet FR also allows us the integration of in vitro gene expression system from single DNA molecules in femtoliter space. A surprising feature of this system is the small variance of gene expression yield, 15% that is remarkably smaller than that of in-cell expression. Taking this advantage, we successfully conducted directed evolutions of enzymes. Arrayed lipid bilayer chamber (ALBiC) displayed the femtoliter reactors, each sealed with a lipid bilayer sheet on top. Single-molecule analysis of membrane transporters was first conducted. Then, to explore the feasibility to form a self-replicating molecular system on the device, we generated a hybrid system of bacterial cell and ALBiC by fusing bacterial protoplast into lipid bilayer. Some of hybrid reactors showed active gene expression and also budding of vesicle structures although in only few cases. In the presentation, I will concisely introduce the latest state of artificial cell reactor project.

O-28

Exploiting nano- and macroscale insights into water-repellent microbes and soil for anticorrosion

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Surface water repellency is a common property of microbial cells, natural and manufactured materials. Insight into the repellency and nanomechanical properties of self-assembling proteins involved (i) in morphological differentiation of *Streptomyces* soil bacteria, and (ii) in natural materials such as soil aggregates, is pivotal to the development of protein applications in materials science, manufacturing and environmental risk management.

This talk will discuss results of interdisciplinary studies into the role of (microbial) proteins in hydrophobicity over length scales ranging from (bio)molecular to nano, and microaggregate to core and manufactured materials scales by applying (ultra)proteomics, quantitative nanomechanical Atomic Force Microscopy, pico- and microliter goniometry, and void network modelling. Approaches into how these unusual proteins can be harnessed, modified, and its anti-corrosive properties monitored at nano- and macroscale will also be discussed.

O-30 (P-87)

Bioinspired sensor material for broad-banded molecular scale, wavelength selective detection

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University of Oxford, UK

Abstract

In the past 50 years, silicon devices such as transistors have been developed, miniaturised and increasingly integrated into numerous items used on a daily basis. Miniaturisation has become the critical step and it is predicted that the size limit of silicon will be reached within the next decade. It is thus necessary to find alternatives, and using functional biomolecules is currently one of the most promising options. Bacteriorhodopsin (bR) is a robust light-driven proton pump found in the purple membrane of the halophilic organism *Halobacterium salinarum*. A photoelectric response of bR was first observed over three decades ago, and the electrical conductance behaviour of the protein has been studied since then. Such studies, though, were only performed within the membrane.

Here, we plan to develop the wavelength tuning potential of bR in electronic devices, either as a photodetector, or as an imaging device, with the molecular scale detector being ~5nm in size, based on our previously published work

Oral Presentations**– 4. Synthetic biology –****O-31 (P-88)****The development of hybrid biomaterials for regenerative engineering**

A. W. Perriman

University of Bristol, UK

Reengineering cells to operate effectively in biological systems invariably involves the assembly of multiple components, which can only be integrated when compatible interfaces are built into the design. This can be achieved through the synthesis of hybrid materials comprising highly cooperative biological and synthetic constituents that can be used to amplify or attenuate host tissue interactions. The systems methodology that underpins this design approach provides a gateway to the development of non-traditional approaches to regenerative medicine. Accordingly, I describe three emerging research programmes that span the fields of synthetic biology, biomaterials, and regenerative engineering: *Stem Cell Painting*¹, where artificial membrane binding proteins undergo spontaneous assembly at the plasma membrane of stem cells; *Cell Paintballing*², where microdroplet vectors are controlled optically to deliver bimolecular payloads to specific membranous regions of individual cells; and *Three-Dimensional Bioprinting*³, where a new hybrid microporous bioink is used to print stem cell laden tissue-like constructs.

1. Armstrong et al., *Nature Communications* **2015**, 6, 7405.

2. Armstrong et al., *Chemical Science* **2015**, 6, 6106.

3. Armstrong et al., *Advanced Healthcare Materials* **2016**, 5, 1724.

Oral Presentations

– 5. Protein-nucleic acid interactions –

O-32

Direct visualization of ParB-DNA interactions using combined Magnetic Tweezers and TIRF microscopy

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The combination of Magnetic Tweezers (MT) with TIRF microscopy allows the simultaneous correlation of mechanical measurements of biomolecules and direct visualization of DNA-protein interactions. The strength of combining these two techniques relies on the advantages they have separately. MT permit the tracking of several DNA molecules in parallel, while a force is applied in a controlled manner. TIRF microscopy exhibits a superior signal to noise ratio over other fluorescence-based techniques, with an evanescent field of a few hundreds of nm from the surface. The drawback is that long DNA molecules need to be stretched across the surface of the flow cell. Here, we present the implementation of a laterally pulling device and its subsequent calibration in flow cells and capillaries. TIRF microscopy was also implemented in this lateral Magnetic Tweezers setup and characterized using fluorescently labelled beads and quantum dots. DNA binding by the *B. subtilis* protein Spo0J/ParB was studied using the combined setup. Fluorescently labelled ParB proteins were able to bind non-specifically along DNA molecules producing a constant emission signal, consistent with the dynamic exchange of protein. Condensation of DNA by ParB was prevented using lateral pulling.

O-34

Structural insights into Endonuclease G in regulating life and death of a cell

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Endonuclease G (EndoG) is an evolutionarily conserved mitochondrial protein that digests nucleus chromosomal DNA during apoptosis and paternal mitochondrial DNA during embryogenesis. EndoG-deficient mice have high levels of mitochondria reactive oxygen species (ROS) and develop heart and mental diseases, showing that the EndoG defect is linked to mitochondrial dysfunction and oxidative stress-related diseases. We reported the crystal structure of the *C. elegans* EndoG homologue, CPS-6, in complex with single-stranded DNA. Two separate DNA strands are bound in the homodimer with their nucleobases pointing away from the enzyme, explaining why CPS-6 degrades DNA without sequence specificity. We also showed that under oxidative stress, homodimeric EndoG becomes oxidized and converts to monomers with diminished nuclease activity. Using *C. elegans* as an animal model, we further show that oxidative stress is linked to cell death defects through the CPS-6-mediated cell death pathway. EndoG homodimer thus plays an unexpected role in acting as a ROS sensor to regulate the life and death of cells. Modulation of EndoG dimer conformation could present an avenue for prevention and treatment of the diseases resulted from oxidative stresses.

O-33

Single-molecule insight into target recognition by CRISPR-Cas enzymes

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Peter Debye Institute for Soft Matter Physics, Universität Leipzig, Germany

The recently discovered CRISPR-Cas enzymes are promising tools in biotechnology and medicine. Central part of these enzyme systems are large effector complexes harboring a short RNA. The RNA promotes recognition of complementary nucleic acid targets by base pairing. The target recognition is, however, highly promiscuous, such that multiple mismatches between RNA and target strand are allowed. This causes technologically undesired off-target binding. Here we investigate the recognition of off-target sites by the CRISPR-Cas surveillance complex Cascade using single-DNA twisting experiments. This allows us to resolve the dynamics of the RNA base-pairing with the target. We find that the target recognition is a dynamic zipping process between RNA and DNA. Upon reaching a mismatch site, the zipping stalls and the intermediate frequently collapses. Eventually thermal fluctuations allow to overcome single mismatches. Upon full zipping of the RNA along the target, a conformational change within the effector complex is triggered that causes DNA degradation. Thus, the complex acts simply as a guard that verifies whether the zipping was successful. Based on these observations we developed a model that can describe the dynamics of the target recognition process.

O-35 (P-96)

Real-time investigation of the assembly dynamics of artificial virus-like particles

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Artificial viruses are model systems for the understanding of natural viruses and potential vehicles for genetic material delivery. It is still a challenge to fully reproduce the natural viral cooperativity behavior during the self-assembly process[1], therefore we are working with simplified model systems in which we can easily and freely tune interaction parameters. This study focuses on the assembly kinetics of an artificial polypeptide designed to self-assemble into a rod-shaped virus-like particle on double stranded DNA[2]. We employed optical tweezers which allowed us to suspend the DNA tether in solution and to monitor by confocal fluorescence microscopy the peptide binding, unbinding and sliding along the DNA in real-time using fluorescently labelled peptides[3]. We also employed acoustic force spectroscopy (AFS) as complementary technique for monitoring the real-time self-assembly at low forces (1-2 pN)[4]. This work opens the doors for new insights into the assembly process of rod-shaped artificial and natural viruses.

[1] *Nature Physics* 2010,6,733-743, [2] *Nature Nanotech.* 2014, 9,698-702, [3] *Methods in Enzymology* 2017,582,85-119, [4] *Nature Methods* 2015,12,47-50

Oral Presentations**– 5. Protein-nucleic acid interactions –****O-36 (P-97)****NMR reveals how phosphorylation of the retinoic acid nuclear receptor regulates gene expression**

D. Martinez-Zapien, C. Köhler, M. Quinternet, I. Lebars, I. Amal, A. Dejaegere, Y. Brélivet, A. Atkinson, M.-A. Del-suc, C. Rochette-Egly, B. Kieffer
IGBMC, France

Nuclear Retinoic Acid Receptors (RARs) are ligand-dependent transcriptional regulators, which mediate the effects of RA, the major active metabolite of vitamin A. RAR shares a common architecture with all other nuclear receptors that includes a ligand binding domain (LBD), a DNA binding domain (DBD) involved in the recognition of hormone specific response elements and an N-terminal intrinsically disordered region (NTD). Recent studies highlighted the importance of the topology of RARs DNA cognate sequences and kinase signalling pathways for fine spatio-temporal regulation of RAR-target genes expression. We have been studying by NMR the molecular mechanism by which the phosphorylation of a conserved serine within the intrinsically disordered region modulates the gene activation of RAR γ . This mechanism involves the phosphorylation-dependent modulation of the affinity between a proline-rich region within the NTD and a SH3 domain of the vinexin β , a RAR γ co-regulator. Our results highlight a complex allosteric mechanism linking RAR γ DNA binding properties with its phosphorylation state at the NTD disordered region. Belorusova AY, *Biochemistry* (2016) 55, 1741

Martinez-Zapien D, et al. (2014) *Protein Expr Purif*, 95C, 113

Lalévée S, et al. (2010) *FASEB J*, **24**, 4523

O-37 (P-98)**Structural information of PTBP1/EMCV complex by combining orthogonal spin labelling with pulse EPR**

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²ETH Zurich, Institute of Molecular Biology & Biophysics, Switzerland

The combination of site-directed spin labeling (SDSL) with pulse Electron Paramagnetic Resonance (EPR) spectroscopy, such as 4-pulse Double Electron-Electron Resonance (DEER), emerged as a promising tool in structural biology to obtain information on site-to-site distances in a range of 1.5 to 8.0 nanometer. Besides the well-established nitroxide radicals, high-spin systems such as Gd(III) based complexes were studied extensively. Combinations of these spin labels turned out to be a powerful technique to get more distance information and thus it offers a valuable method for unknown structures.

Here, we present a calibration method for different spin labels by measuring protein-protein distance distributions in individual RNA binding domains of the alternative splicing regulator Polypyrimidine-Tract Binding Protein 1 (PTBP1). For our system, the complex of PTBP1 and the Encephalomyocarditis Virus (EMCV) RNA, we follow the approach of orthogonal SDSL to obtain structural information in terms of orientation and arrangement by attaching Gd(III)-based spin labels on the protein and nitroxide radicals on the RNA. We aim then to combine short-range NMR restraints with long-range EPR restraints to determine the 3-dimensional structure of this protein/RNA complex.

Oral Presentations

– 6. Protein structure to function –

O-38

Dynamic complexes and complex dynamics: from fundamental biophysics to physiological function

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Protein dynamics lie at the origin of biological function. Intrinsically disordered proteins (IDPs) represent extreme examples where flexibility defines the molecular paradigm. To understand their function, we need to describe the conformational energy surface¹ and associated dynamic timescales. We recently used multi-field, temperature-dependent NMR relaxation to reveal distinct dynamic modes occurring on timescales over 3 orders of magnitude.²

Despite their ubiquity, the mechanisms regulating IDP function remain poorly understood. We use exchange-NMR to describe molecular recognition trajectories from free-state to the bound state ensembles. Examples include the replication machinery of paramyxoviruses,³ specificity in the JNK signalling pathway,⁴ or ultra-weak interactions facilitating fast passage of cargo through the nuclear pore.⁵

Finally, a combination of NMR, smFRET and SAS reveals that large-scale domain dynamics, mediated by a linker region undergoing an order-to-disorder transition, allow the C-terminus of Influenza polymerase PB2 to adopt an open conformation that is essential for import into the nucleus.⁶

¹ Jensen et al *Chem Rev* 2014; ² Abyzov et al *JACS* 2016;

³ Schneider et al *JACS* 2015; ⁴ Kragelj et al *PNAS* 2015;

⁵ Milles et al *Cell* 2015; ⁶ Delaforge et al *JACS* 2015

O-40 (P-129)

Bacterial surface-layer-protein assemblies at atomic scale

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S-layers are (glyco)protein coats displayed on the external surface of many bacterial and archaeal species. As the outermost layer, the S-layer is in direct contact with the environment and thus involved in e.g. adhesion to epithelial cells and intestinal components. S-layers however remain poorly understood, primarily due to lack of structural knowledge.

We characterized the bacterial surface layer proteins SbsC from *Geobacillus stearothermophilus* and SlpA of *Lactobacillus acidophilus* and *L. amylovorus*. Several soluble fragments as well as full-length proteins assembling to 2D-crystals were produced. To elucidate the structure of the complete S-layers, an integrative structural biology approach combining X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR), mass spectrometry and electron microscopy has been applied. To additionally characterize the binding of S-layer proteins to the bacterial cell wall we performed isothermal titration calorimetry (ITC) experiments. The obtained results allow us to learn more about the cell wall attachment and self-assembly formation on atomic scale.

O-39

MicroED opens a new era for biological structure determination

T. Gonen

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My laboratory studies the structures of membrane proteins that are important in maintaining homeostasis in the brain. Understanding structure (and hence function) requires scientists to build an atomic resolution map of every atom in the protein of interest, that is, an atomic structural model of the protein of interest captured in various functional states. In 2013 we unveiled the method MicroED, electron diffraction of microscopic crystals, and demonstrated that it is feasible to determine high-resolution protein structures by electron crystallography of three-dimensional crystals in an electron cryo-microscope (CryoEM). The CryoEM is used in diffraction mode for structural analysis of proteins of interest using vanishingly small crystals. The crystals are often a billion times smaller in volume than what is normally used for other structural biology methods like x-ray crystallography. In this seminar I will describe the basics of this method, from concept to data collection, analysis and structure determination, and illustrate how samples that were previously unattainable can now be studied by MicroED. I will conclude by highlighting how this new method is helping us understand major brain diseases like Parkinson's disease.

O-41 (P-130)

Observation of water-channel opening of cytochrome c oxidase by time-resolved XFEL crystallography

M. Kubo¹, A. Shimada², S. Baba³, K. Yamashita¹, K. Hirata¹, M. Yamamoto¹, K. Shinzawa-Itōh², H. Ago¹, S. Yoshikawa², T. Tsukihara²

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Bovine heart cytochrome *c* oxidase (CcO) is a large membrane enzyme (210 kDa) that catalyzes O₂ reduction to water, coupled with proton pump across the mitochondrial inner membrane. The enzyme includes a water channel and a hydrogen-bond network in tandem as a proton-pump pathway. After the O₂ reduction at the active center consisting of Fe_{a3} and Cu_B, CcO receives four electron equivalents from cytochrome *c*. Each of the electron transfer processes is coupled with pumping of one proton equivalent, during which the water channel is closed to prevent proton back leak. Recently, it was revealed that binding of CO (O₂ analogue) to Fe_{a3} induces a bulge (unpaired backbone C=O) formation at Ser382 in Helix-X, which closes the water channel. To elucidate the water-channel gating mechanism, here, we developed an instrument for time-resolved X-ray crystallography using an X-ray free electron laser at SACLA, and investigated the structural dynamics upon CO photo-dissociation from Fe_{a3}. As a result, we successfully observed the water channel opening processes, accompanied with transient CO binding to and dissociation from Cu_B. The water-channel gating mechanism based on a dynamic interaction between the active center and Helix-X will be discussed.

Oral Presentations

– 6. Protein structure to function –

O-42 (P-131)**A tri-ubiquitin bridges two ABIN2 dimers to form a higher-order signaling complex**Y.-C. Lo^{1,2}, S.-M. Lin¹, S.-C. Lin³

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Poly-ubiquitin chains are important signaling molecules that were assembled by the ubiquitination process in order to drive different linkage-specific signaling pathways through subsequent protein-protein interaction with downstream signaling molecules. For example, K63 and linear poly-ubiquitin chains play an important role in NF- κ B activating pathway. A20 could down regulate the NF- κ B activation pathway by interacting and degrading the poly-ubiquitin chains. The interaction is mediated by ABIN2, which has both A20- and linear poly-ubiquitin interacting domains. How a poly-ubiquitin chain interacts with signaling molecules remains largely unknown. Here we report the crystal structure of an ABIN2:tri-Ub complex. ABIN2 has a primary and a secondary linear-Ub binding site. Surprisingly, a tri-Ub molecule could simultaneously interact with two ABIN2 dimers, in which the ubiquitins form a helical trimer when bridging two hABIN2 dimers. Our studies suggest the formation of a higher-order complex between linear poly-polyubiquitin chain, ABIN2, and A20.

Oral Presentations

– 7. Nanobiophysics –

O-43

Linking mechanochemistry with protein folding with single bond resolution

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The nanomechanical properties of elastomeric proteins determine the elasticity of a variety of tissues. Post-translational modifications (PTMs) have recently emerged as a useful tactic to regulate protein nanomechanics. The formation of disulfide bonds is a widespread natural strategy to regulate protein stiffness. Here we propose two distinct chemical routes to promote non-enzymatic oxidative protein folding through the reactivity of selected PTMs. Using single-molecule force-clamp spectroscopy, DFT calculations and mass spectrometry, we first captured the reactivity of an individual sulfenic acid PTM. Our results demonstrated that sulfenic acid is a crucial short-lived intermediate that dictates the protein's fate in a conformation-dependent manner. A second method to induce disulfide formation occurs via isomerization of naturally occurring thiols. We showed that subtle structural changes in a mixed-disulfide intermediate between a protein cysteine and an attacking low molecular-weight thiol have a dramatic effect on the protein's mechanical stability. Combined, these chemistry-based mechanisms for non-enzymatic oxidative folding provide a plausible explanation for redox-modulated stiffness of proteins that are physiologically exposed to mechanical forces such as cardiac titin

O-45

Single-molecule label-free analysis of sequence specific protein-DNA interactions

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Protein interactions with specific DNA sequences are crucial in the control of gene expression and the regulation of replication. Single-molecule methods offer unique capabilities to unravel the mechanism and kinetics of these interactions. Here we develop a nanopore approach where a target DNA sequence is contained in a hairpin followed by a ssDNA. This system allows DNA-protein complexes to be distinguished from bare DNA molecules as they are pulled through a single nanopore detector, providing both the equilibrium and kinetic constants of the interactions. We show that this approach can be used to test the inhibitory effect of small molecules on complex formation, and their mechanisms of action. In a proof of concept, we use DNAs with different sequence patterns to probe the ability of the nanopore to distinguish the effects of an inhibitor in a complex mixture of target DNAs and proteins. We anticipate that the use of this technology with arrays of thousands of nanopores will drive the discovery of new inhibitors of transcription factor binding.

O-44

Ion channels and porins made from DNA

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DNA nanotechnology has enabled the construction of DNA origami nanopores [1]. These synthetic designer nanopores promise improved capabilities for enhanced single molecule detection. Here, we will review the recent developments of DNA origami nanopores in lipid membranes [2]. These structures have extraordinary versatility and are a new and powerful tool in nanobiotechnology for a wide range of important applications. In this talk I will discuss the recent realisation of ion channel entirely made from DNA [3,4]. The versatility of the DNA nanotechnology enables the creation of designer nanopores as model systems for protein channels from channels to porins spanning several orders of magnitude in conductance and molecular weight [4,5].

[1] Bell, *et al.* DNA origami nanopores. *Nano Letters*, 12(1):512-517, 2012

[2] Burns, *et al.*, *Angewandte Chemie*, 52(46):12069-12072, 2013

[3] Goepfrich, *et al.* *Nano Letters*, 15(5):3134-3138, 2015

[4] Goepfrich, *et al.* *Nano Letters*, 16(7):4665-4669, 2016

[5] Goepfrich, *et al.* *ACS nano*, 10(9):8207-8214, 2016

O-46 (P-262)

Probing early virus binding steps towards living cells using force-distance curve-based AFM

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Université Catholique de Louvain, Institute of Life Sciences, Louvain-la-Neuve, Belgium

Currently, there is a growing need for methods that can quantify and map the molecular interactions of biological samples, both with high-force sensitivity and high spatial resolution. Force-distance (FD) curve-based atomic force microscopy is a valuable tool to simultaneously contour the surface and map the biophysical properties of biological samples at the nanoscale. This presentation reports the use of advanced FD-based technology combined with AFM tips functionalized with single virions to probe the localization of specific binding sites on living cells at high-resolution. We also introduce experimental and theoretical developments that allow force-distance curve-based atomic force microscopy (FD-based AFM) to simultaneously image cell surfaces and to quantify their dynamic binding strength to single virus particle. These binding strengths provide kinetic and thermodynamic parameters of individual ligand-receptor complexes and shed new light into the understanding of how viruses exploit fundamental cellular processes to gain entry to cells and deliver their genetic cargo.

Oral Presentations**– 7. Nanobiophysics –****O-47 (P-261)****Using AFM to study red blood cells' morphology and elasticity on Amyotrophic Lateral Sclerosis**C. S. Lopes¹, A. C. Pronto-Laborinho^{1,2}, M. de Carvalho^{1,2}, N. C. Santos¹, F. A. Carvalho¹¹Instituto de Medicina Molecular, Lisbon, Portugal; ²Serviço de Neurofisiologia, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisbon, Portugal

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease. Patients' complications promote changes in hemodynamic properties and abnormalities in red blood cells (RBC) membrane and on its lipid content. Our main goal was to evaluate changes in the viscoelastic and morphological properties of RBCs in ALS. Blood samples from ALS patients were compared with healthy donors. By Atomic Force Microscopy, RBCs' membrane roughness, elasticity and morphological parameters were analysed for both groups. Patients' RBCs are stiffer, have higher penetration depth and are more capable to deform. RBCs from ALS patients are thicker and have a higher cell area. Zeta-potential analysis showed that RBC membranes from ALS patients are less negatively charged, which may be due to a lower density of sialic acid residues on the RBC surface. Patients' RBC membranes have an increased roughness. ALS patients RBC membranes, assessed by fluorescence spectroscopy, are more fluid. This may be associated with changes on membrane lipid composition and packing. We conclude that RBCs from ALS patients present significant electrostatic and morphologic changes on their membranes. These findings may contribute to understand the complex interplay between disease progression rate and RBCs lipid profile.

O-48 (P-260)**The mechanical properties of HIV-1 capsid during reverse transcription: insights into uncoating**S. Rankovic¹, J. Varadarajan², R. Ramalho¹, C. Aiken², I. Rouso¹¹Ben-Gurion University of the Negev, Israel; ²Vanderbilt University Medical Center, USA

For successful infection, the HIV-1 genome, which is in the form of a single-stranded RNA enclosed inside a capsid shell, must be reverse transcribed into double-stranded DNA and released from the capsid (in a process known as uncoating) before it can be integrated into the target cell genome. Although HIV-1 uncoating has been linked to reverse transcription of the viral genome in target cells, the mechanism by which uncoating is initiated is unknown. Using time-lapse atomic force microscopy, we analyzed the structure and physical properties of isolated HIV-1 cores during the course of reverse transcription *in vitro*. We find that, during reverse transcription the pressure inside the capsid increases, reaching a maximum after 7 hours. High-resolution mechanical mapping reveals the formation of a coiled filamentous structure underneath the capsid surface. Subsequently, this coiled structure disappears, the stiffness of the capsid drops precipitously to a value below that of a pre-reverse transcription core, and the cores partially or completely rupture. We propose that the transcription of the relatively flexible ssRNA into the more rigid RNA-DNA hybrid elevates the pressure within the core, which induces uncoating.

Oral Presentations**– 8. Forces in and between cells: filaments, membranes and walls –****O-49****Mechano-devo: how growth and form derived mechanical forces channel morphogenesis**

O. Hamant

INRA, ENS Lyon, France

In “On growth and forms” (1917), D’Arcy Thompson stresses the inevitable interactions between physics and biology. Thanks to ongoing developments in live imaging and modeling, this field of study has been rejuvenated: the relation between mechanics and shape changes can now be addressed more comprehensively, notably in plants in which morphogenesis is mainly determined by cell walls. In past work, we showed that shape- and growth-derived forces act as signals that orient plant microtubules (Hamant *et al.*, 2008 Science). This response channels key biological features, such as cell shape (Sampathkumar *et al.*, 2014 eLife), cell division plane orientation (Louveau *et al.*, 2016 PNAS) and final organ shape (Hervieux *et al.*, 2016 Curr. Biol.). Beyond microtubules, such forces also contribute to cell polarity (Heisler *et al.*, 2010 Plos Biol.) and to the expression patterns of master regulators of meristem maintenance (Landrein *et al.*, 2015 eLife). The implications of this work are numerous and include a role of mechanical conflicts emerging from growth heterogeneity in the reproducibility of shapes. Altogether, this provides a picture in which mechanical forces add robustness to plant morphogenesis, by channeling the dynamics of cell effectors and molecular pathways.

O-51**Sensing matrix rigidity: transducing mechanical signals from integrins to the nucleus**P. Roca-Cusachs^{1,2}¹Institute for Bioengineering of Catalonia, Spain; ²University of Barcelona, Spain

Cell proliferation and differentiation, as well as key processes in development, tumorigenesis, and wound healing, are strongly determined by the rigidity of the extracellular matrix (ECM). In this talk, I will explain how we combine molecular biology, biophysical measurements, and theoretical modelling to understand the mechanisms by which cells sense and respond to matrix rigidity. I will discuss how the properties under force of integrin-ECM bonds, and of the adaptor protein talin, drive and regulate rigidity sensing. I will further discuss how this sensing can be understood through a computational molecular clutch model, which can quantitatively predict the role of integrins, talin, myosin, and ECM receptors, and their effect on cell response. Finally, I will analyze how signals triggered by rigidity at cell-ECM adhesions are transmitted to the nucleus, leading to the activation of the transcriptional regulator YAP.

O-50**Forces in plant cell walls during growth and under stress conditions**

K. Radotić

Institute for multidisciplinary research, University of Belgrade, Serbia

Plant cell wall mechanics and its relationship to growth and environmental stress are not entirely revealed. By using atomic force microscopy stiffness tomography we monitored stiffness distribution in the cell wall layers and its evolution during growth phases of living single Arabidopsis cells. In the beginning and end of cell growth, the average stiffness of the cell wall was low and the wall was mechanically homogenous, while in the exponential growth phase the average wall stiffness increased, with increasing heterogeneity and polysaccharide/lignin content shown by Fourier-transform infrared (FTIR) spectroscopy. In adult plants, we studied mechanical stimuli- induced compression/tension reaction in the tree stems, and thigmotropic twining of the vines. Confocal- and fluorescence detected linear dichroism (FOLD) microscopy revealed that in juvenile spruce stems under bending stress, cell wall organization- changed lignin structure and cellulose fibril orientation was more precise indicator of compression severity than compression wood amount. Our spectroscopic, FTIR- and FOLD microscopic studies of parenchyma cell walls in Dioscorea vine stem mechanics showed that mechanical strain changes polysaccharide/lignin ratio and organisation, cellulose fibril order staying unchanged.

O-52 (P-320)**Bulk cytoplasmic actomyosin contractions drive streaming in zebrafish eggs**

S. Shamipour, B. Hof, C.-P. Heisenberg

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At the onset of zebrafish development, the fertilized egg is composed of a mixture of yolk granules (the food supply on which the developing embryo will feed on) and cytoplasm from which all of the embryonic tissues will develop. For development to start, the cytoplasm and yolk granules need to segregate with all the cytoplasm accumulating at one side of the oocyte (animal pole) and the yolk granules on the other (vegetal pole). This segregation process is typically called ‘cytoplasmic streaming or flow’ and the goal of this project is to unravel the physical basis of cytoplasmic streaming within the oocyte.

Previous studies have speculated that reorganization of cortical actomyosin triggers cytoplasmic flows within the oocyte. However, by generating oocytes specifically lacking cortical actomyosin, we were able to show that cytoplasmic streaming also occurs in the absence of the cortical actomyosin network. Instead, we propose that contraction of a previously uncharacterized subcortical actomyosin network can drive this process.

Oral Presentations

– 8. Forces in and between cells: filaments, membranes and walls –

O-53 (P-321)**Correlative AFM and cryo-EM approach for probing the nuclear lamina mechanics**T. Sapra¹, Z. Qin², M. Buehler², O. Medalia¹¹University of Zurich, Switzerland; ²Massachusetts Institute of Technology, USA

Nuclear lamina forms a shell-like structure in the nucleus. Mutations in the lamin proteins cause disease collectively termed laminopathies. We have developed an approach to study the *in situ* mechanics of nuclear lamins (*Xenopus laevis* oocyte nucleus) at the single filament level, to our knowledge for the first time. This is also the first example of combining AFM and cryo-electron tomography to understand the structural framework of the lamin network, an important step toward combining mechanobiology and visual proteomics. The combination of structural mechanics and network theory analysis provides a credible analysis of the hierarchical organization of the nuclear lamina. The results are in good agreement with molecular dynamics simulations. The study paves the way for further studies of nuclear mechanics in mammalian nuclei.

O-54 (P-322)**Stiffening and softening of cytoskeletal networks: rheological insights from minimal systems**

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The cytoskeleton is impressively versatile, manifesting the ability to either stiffen or soften in different stress regimes. A major challenge in cell mechanics has been to identify the mechanistic origin of this behaviour. We address this challenge by preparing minimal in-vitro systems of intermediate filaments and microtubules and probing their mechanical response over different applied stress regimes.

Intermediate filament networks are probed rheologically, increasing the mechanical load with intermittent constant stress. This allows us to probe mechanical response in new high-stress regimes and also to quantitatively measure the network's remodelling. We reveal a rich diversity of mechanical responses with stiffening at low forces, axial stretching at intermediate forces and a new regime of network softening at high forces.

Through analysis of the network strain over time and by comparing permanent and transient crosslinking agents we reveal that remodelling is driven by crosslinker unbinding and loss of network connectivity. We also present new findings on how the inclusion of microtubules can modulate the onset of these different mechanical regimes, providing new insights into how cells are able to store and dissipate applied mechanical loads.

Oral Presentations

– 9. Systems biology –

O-55

Inferring cause and effect in complex molecular systems from image fluctuations

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One of the major limitations in the study of complex molecular pathways is adaptation of the system to experimental perturbation. With ‘complex’ we mean pathways with a significant level of functional redundancy between components and nonlinear interactions. In this scenario, perturbation of one component may lead to an observable phenotype. However, it is impossible to interpret the difference between phenotype and wildtype in terms of the function the targeted component fulfills in the unperturbed system – although it is the predominant approach cell and systems biologists take to dissect molecular functions. Over the past decade my lab has made efforts to circumvent this problem by exploiting basal fluctuations of molecular systems in order to establish causal functional relations between pathway components. Inspired by the field of econometrics, where predictive models are built entirely from passive observation of financial fluctuation time series, we have developed a novel mathematical framework to determine nonlinear interdependencies between molecular components. I will introduce the mathematical, computational, and experimental concepts based on which we can now accurately delineate the functional hierarchy between signaling and mechanical processes driving cell protrusion events as a prime example of a complex molecular process.

O-57 (P-329)

Applications of stochastic lumping analysis to fluctuations in systems and structure biology

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Conventional studies in systems and structure biology rely largely on the construction of kinetic schemes. Since the selection of these networks depends on the level of coarse-graining and thus is not unique, a concern is raised whether and under which conditions hierarchical schemes can reveal the same experimentally measured fluctuating behaviors and unique fluctuation related biological properties. To clarify these questions, we introduce stochasticity into the traditional lumping analysis, generalize it from rate equations to chemical master equations and stochastic differential equations, and extract the fluctuation relations between kinetically and thermodynamically equivalent networks under intrinsic and extrinsic noises. The results provide a theoretical basis for the legitimate use of low-dimensional models in the studies of biological fluctuations and, more generally, for exploring stochastic features in different levels of contracted networks in chemical and biological kinetic systems. [J. Chem. Phys. 142, 184103 (2015)]

O-56

Yeast mating in space and time

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Baker's yeast (*Saccharomyces cerevisiae*) has a diploid lifestyle, but under stress conditions it forms spores which then release haploid cells of mating types *MATa* and *MAT α* . *MATa* cells are a frequently used model organism for many cell biological studies of cell cycle, metabolism or signaling. *MATa* and *MAT α* cells can also mate to form diploid cells again. To this end, they secrete the pheromones α -factor and α -factor, sense the opposite pheromone and form protrusions in the direction of a potential mating partner. Importantly, they cannot move towards their mating partner, thus, the formation of the mating shape called shmoo is a significant investment.

Combining experimental studies of the cellular responses to mating factor and the resulting shape changes with spatial mathematical modeling, we investigated three major steps in the mating process. Specifically, we asked (i) how yeast cells communicate to form sharp gradients allowing precise decisions, (ii) how the individual cells sense the resulting gradients and (iii) how they translate the sensed information into shape changes.

We found that success in mating is a community property, although individual cells have to decide whether to engage in mating. Using biophysical principles, we could also elucidate the processes required to enable the necessary directionality decisions and shape changes.

O-58 (P-330)

Escherichia coli's strategies for maintaining proton motive force when exposed to photodamage

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Fluorescent microscopy is a powerful tool for visualization and dynamic observation of cell's characteristics (size, shape, internal pH *etc.*). However, repeated exposure of fluorescently labeled cells to illumination from lasers or high-intensity lamps and LEDs can lead to photodamage and changes in cell physiology. The damage is attributed to reactive oxygen species (ROS) formed during cell exposure to light. It has been shown that ROS formation inhibits cell growth, damages the membrane and causes DNA breaks.

Despite these findings, the immediate response of live cells to photodamage has not been identified. Here we combine epi-fluorescence and back focal plane interferometry to simultaneously monitor, in individual cells, two components of *Escherichia coli*'s energetics – proton motive force (PMF) and internal pH. We demonstrate that under photodamage *E. coli* cells lose PMF in a fashion analogous to a capacitor discharging if kept in nutrient deprived buffer. Interestingly, in nutrient rich media, *E. coli* maintains, or even increases, the PMF in response to photodamage at the expense of severe loss of internal pH. We present a mathematical model that offers a possible explanation for our observations and discuss the model predictions for the overall cell energy maintenance.

Oral Presentations**– 9. Systems biology –****O-59 (P-331)****Chemically-driven kinetics of phase separated membrane-free organelles**

J. D. Wurtz, C. F. Lee

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Liquid-liquid phase separation of proteins from cytoplasm allows the cell to spatially organise its interior by assembling membrane-free, liquid organelles. In equilibrium fluids, a collection of droplets is thermodynamically unstable and coarsen via Ostwald ripening and coalescence. In the non-equilibrium cellular environment, it is unclear how membrane-free organelles are stabilized. We study theoretically the effect of non-equilibrium chemical reactions on a phase separated ternary mixture, composed of a two-state protein and cytoplasm. One state is prone to phase separate, the other state is soluble, and first order chemical reactions convert one state into the other, and vice versa. By controlling the reaction rates, one can bring the system into a stable, monodisperse droplet regime, where Ostwald ripening is arrested, or into an unstable regime with active Ostwald ripening. We predict a relationship between the reaction rates, and protein concentration gradients inside and outside droplets, and the droplet sizes. Finally, we apply our results to stress granules, a class of membrane-free organelles that form during various environmental stresses.

Oral Presentations

– 10. Quantum biology –

O-60

Photon and electron counting statistics of single photosynthetic complexes

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It is well known that the primary steps of photosynthesis rely on quantum mechanical phenomena. For instance, excitons or the collective electronic excitations of pigment-protein complexes are a clear manifestation of collective quantum behaviour and the formation of such excitons is essential for efficient absorption of sunlight by photosynthetic organisms. However, when it refers to excitation energy distribution and conversion in the picosecond time scale, it is not entirely clear which dynamical features can only be predicted within a quantum mechanical framework or how such quantum features affect the biological function of photosynthetic complexes. In this talk, I will present our research on the quest of signatures of quantum coherent process in photosynthetic complexes with a focus on the fingerprints such phenomena may leave in photon and electron counting statistics experiments. Our work shows that frequency-filtered photon statistics could provide unambiguous signatures of coherent interactions between electronic and vibrational degrees of freedom in light-harvesting complexes and that electric current fluctuations could provide information on the functional role of the vibrational motions assisting energy and charge transfer in photosynthetic reaction centres.

O-62 (P-346)

Quantum vibrational excitations and protein folding *in vivo*

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One main hypothesis is that when triggers like water molecules or ions or other ligands, or chemical reactions, act on a protein, the energy input to the protein is in the form of local quantum vibrational excited states (the VES hypothesis). Simulations show that, **as quantum states**, these VES can hop **without dissipation**, from the active site, where they are generated, to other sites in the proteins. This presentation is concerned with the application of the VES hypothesis to protein folding. According to mainstream theories, the native state of proteins is that which minimizes its (free) energy. Instead, the view here is that the native state is just one of the many stable kinetic traps into which the same protein may fall. In this perspective, protein folding is a kinetic process and folding reproducibility requires not only that the pathway followed by the protein from the initial conformation is always the same, but also that the initial conformation is always the same. My hypotheses are that the nascent chain of **all** proteins is helical and that the first step in protein folding is the bending of that initial helix at specific sites. It is shown that the application of the hypotheses described above can lead to a complete picture of the folding of a small all-alpha protein.

O-61

Electron-vibrational coupling: from natural and artificial light-harvesting to olfaction

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The coupling of vibrational motion, coherent and incoherent, to excitonic and electronic dynamics is a central element of our understanding of excitation energy and charge transport as well as charge separation dynamics in natural and artificial light-harvesting systems [1] and has potential implications on our understanding of olfaction. In this talk I will discuss the concept of noise-assisted transport [2] and the proposal of the central role of the coupling of long-lived vibrations and electronic motion to long-lived coherent signals and transport [3]. I will report both theoretical work and recent experimental efforts towards the demonstration of vibronic coupling in artificial light harvesting [4, 5] and explain how electron-vibrational coupling may provide an alternative theory of odor recognition based on vibrational spectra of olfactants [6].

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O-63 (P-567)

Quantum calculations on the voltage sensing domain (VSD) of the Kv1.2 potassium channel

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A 976 atom subset of the Kv1.2 channel voltage sensing domain (with water) was optimized at Hartree-Fock level. Side chains pointing away from the center of the VSD were truncated; S1,S2,S3 end atoms were fixed; S4 end atoms were fixed in some calculations, free in others. Following optimization, single point calculations (B3LYP/6-31G**) allowed accurate comparisons of energies. Open conformations (*i.e.*, membrane potentials ≥ 0) are consistent with the known X-ray structure of the open state when some salt bridges in the VSD are not ionized (H^+ on the acid, S4 end atoms fixed or free). The backbone of the S4 segment, free or not, moves no more than 2.5 Å upon switching from positive to negative membrane potential, and the movement is wrong for closing the channel. This leaves H^+ motion as gating current. Groups of 3-5 side chains are important for proton transport, based on the calculations. A pair of steps: proton transfer from a tyrosine, Y266, through arginine (R300), to a glutamate (E183), is a key result, providing approximately 25% of gating current. Calculated charges on each arginine and glutamate are appreciably less than one. The calculation predicts that a Y266F mutation would have a drastic effect on the channel, possibly killing it. Alternate interpretations of experiments usually understood in terms of the standard model are shown to be plausible.

Oral Presentations

– 11. Computational biophysics –

O-64

Enhanced sampling approaches for cryptic site discovery

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“Cryptic” pockets, that is, sites on protein targets that only become apparent when a drug bind, offer an attractive opportunity for the development of allosteric drugs for difficult targets. However, due to their “hidden” nature, they have been in most cases discovered serendipitously. What is more, the molecular mechanism by which cryptic sites are formed is not clear. We used a number of enhanced-sampling simulation approaches [1] with different state-of-the-art force-fields to investigate the nature of cryptic sites in various pharmacologically relevant targets. The mechanisms of cryptic site formation that we observe is suggestive of an interplay between induced-fit and conformational selection. Employing this insight, we developed a novel approach “SWISH” (Sampling Water Interfaces through Scaled Hamiltonians) [2] and used it in combination with Metadynamics to understand the binding mode of a novel allosteric modulator of the extracellular portion of the Fibroblast Growth Factor Receptor [3].

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O-66

Structural ensembles of intrinsically disordered proteins using all-atom molecular simulation

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There is increasing interest in intrinsically disordered peptides and proteins (IDPs) due to their abundance and functional importance, as well as their association with various human diseases. Instead of folding into a single, well-defined 3D structure, an IDP fluctuates between an ensemble of interconverting conformations. Molecular simulations can now reach relevant timescales for sampling IDP conformations, but a major limiting factor lies in the accuracy of their underlying empirical force fields (FFs). Protein FFs were developed primarily targeting folded proteins and their accuracy in modelling IDPs needs to be scrutinized and improved. We carried out a benchmark study on the structural ensembles of an IDP obtained with eight different force fields. The CHARMM36 (C36) protein FF was found to generate a high population of left-handed α -helix (α L), inconsistent with experimental measurements. We report an improved version of the C36 FF, C36m, that overcomes this bias towards α L-helix. Extensive validation simulations illustrate that the improved force field is suitable for MD simulations of both folded and disordered proteins.

O-65

Modeling experiments in simulations and simulations in experiments: Combining modeling with low and medium resolution structural biology

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Modeling and simulations of complex biological macromolecules has made tremendous progress since the 1960s as the result of a wonderful marriage of theoretical and computational advances. However, while molecular dynamics simulations have been very powerful for exploring and explaining the atomic detail of processes, with a few exceptions it is not until the last few years these methods have become predictive enough to compete with experimental techniques. I will discuss some of the work we (and other teams) are doing to integrate experimental data earlier in theoretical studies. In particular,

I will show how we try to combine molecular dynamics simulations e.g. with low-resolution data from electrophysiology and structural biology to create models of gating and allosteric modulation in ion channels. I will also present recent work where we are developing new methods for very rapid reconstruction of three-dimensional electron densities from cryo-EM data, and introduce a new framework for using restraints from multiple sources of possibly conflicting experimental measurements to efficiently refine structures in molecular simulations.

O-67 (P-350)

Codon recognition on the ribosome - free energy and QM/MM calculations

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Classical free energy calculations have been combined with QM and QM/MM calculations of the affinity of codon-anticodon interactions in the ribosomal decoding site, where several crystal structures [1,2] indicate that an anticodon base in the wobble position, assumes the normally high-energy enol form when it is involved in a non-cognate base pair. Our previous classical free energy calculations[3], which do not account for the cost of the keto→enol conversion, showed that the extra hydrogen bond provided by the enol form would indeed stabilize the non-cognate base pairs. We have now extended these results with QM/MM calculations in the ribosomal context.

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Oral Presentations**– 11. Computational biophysics –****O-68 (P-351)****Structural dynamics of monomeric alpha-synuclein on the ps-μs time scale derived from MD simulations**

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α-synuclein[aS] is an intrinsically disordered protein[IDP], which can form pathogenic β-sheet aggregates. A number of dynamically reordering regions exist, in which secondary structure elements form and melt on ns-μs timescale. Here we used molecular simulations to quantify the timescales at which the aS monomer reorders. Both structure and dynamics of IDPs are very sensitive to force field inaccuracies. We therefore performed extensive test simulations using four force field /water model combinations and compared the results to NMR, SAXS and FRET data. We find that secondary structure forms and dissolves on time scales of a few μs, very similar to those of tertiary structure rearrangements. Remarkably, both largest β-sheet formation rates and propensities were seen for the NAC region, involved in fibril formation. Since the on-rate for β-sheet aggregation is much slower, a conformational selection mechanism seems plausible for aggregation. We also investigated the spontaneous α-helix formation in the structurally variable region between residues 1-100. The on-rates for α-helix formation are very low or not observed at all. This suggests an induced fit or much slower conformational selection mechanism for α-helix formation upon membrane binding compared to β-sheet formation.

O-69 (P-352)**Getting the ion-protein interactions right in Molecular Dynamics simulations**

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Ion-biomolecules interactions are ubiquitous and play a central role in a number of fundamental biological processes, such as calcium signaling and insulin storage in the ion-rich secretory granules. However, assessing the ion-biomolecule interactions is difficult because of the complexity of the systems involved: quantum descriptions are too expensive to study such large systems, and solvation properties of divalent cations are poorly reproduced by standard force fields, because of polarization and charge transfer effects.

Our goal is to improve the description of cations in simulations and apply it to biologically relevant problems. We obtain reference data for the interaction of non transition metal divalent cations (Ca²⁺, Mg²⁺, Zn²⁺) with typical protein groups on small model systems, which we study combining *ab initio* Molecular Dynamics simulations and neutron-scattering experiments. This leads to the development of a scaled charge description of the ions, which takes into account electronic polarization in a mean field way [1]. The obtained force field is applied to the interaction of ions with the insulin molecule in different multimeric states.

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Oral Presentations**– 12. Protein misfolding –****O-70****Assignment and atomic-resolution structure of an A β (1–42) amyloid fibril**A. Böckmann¹, B. H. Meier², M. A. Wälti², F. Ravotti², P. Güntert³, R. Riek²¹CNRS/Université de Lyon, France; ²ETH Zurich, Switzerland; ³Goethe University Frankfurt am Main, Germany

Amyloid- β (A β) is involved in Alzheimer's disease, and is found in plaques in human brain containing the metabolic product of the amyloid precursor protein (APP). Although the two major forms A β (1–40) and A β (1–42) differ in only two residues, they display different biophysical, biological, and clinical behavior. Here, we assigned all 38 residues which show visible resonances in the spectra using 3D solid-state NMR methods. We compare these chemical shifts to those obtained by other groups. Based on distance measurements using PAR, DARR and CHHC spectra on diluted and fully labeled samples, we determined the 3D fold of the fibrils by using unambiguous and low ambiguous restraints and their combination with mass-per-length measurements from electron microscopy. Based on this fold, we used an automated structure calculation using all restraints extracted from the spectra. Residues 15–42 form a rigid cross- β -sheet entity with maximally buried hydrophobic side-chains. Residues 1–14 are partially ordered and in a β -strand conformation, but do not display unambiguous distance restraints to the remainder of the structure. We compare the structure to the one of other A β (1–42) preparations described in the literature and to the Osaka mutant A β (1–40) E22A.

O-72**Secondary nucleation in amyloid formation**

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The assembly of proteins into amyloid fibrils is a central phenomenon in several human diseases. We use a combination of NMR, optical spectroscopy and mass spectrometry to study the mechanism of peptide self-assembly into amyloid fibrils. Our studies are focused on amyloid β peptide (A β) from Alzheimer's disease and α -synuclein (α -syn) from Parkinson's disease. The goal is to find the underlying microscopic steps in the aggregation process and their molecular driving forces. We study the process starting from pure monomers, or monomer supplemented with a defined amount of pre-formed fibrils. We also study monomers mixtures including length variants, or monomers supplemented with inhibitors or bilayer membranes in the form of with phospholipid vesicles. The results reveal for both proteins the importance of surface catalyzed secondary nucleation of monomer on fibril surface. In particular, we have found that secondary nucleation of monomers on the surface of existing aggregates is the dominant nucleation mechanism for A β at several solution conditions, including neutral pH and physiological salt, and for α -syn at mildly acidic pH relevant for endosomes and other intracellular compartments. We investigate the molecular determinants of secondary nucleation and have found that very high level of specificity.

O-71**Molecular mechanisms of neurodegeneration and therapeutic intervention**

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Understanding the formation and propagation of aggregates of the Alzheimer disease-associated Tau and Amyloid β protein *in vivo* is vital for the development of therapeutics for this devastating disorder. Using two colour super-resolution imaging we show that there is heterogeneity in the growth rates of individual amyloid fibrils which can be attributed to a structural polymorphism. We have developed this technique further for its application in samples of CSF and interstitial fluid from patients. Furthermore, using our aggregation sensor, we demonstrate that we can follow amyloid formation and propagation in live neuronal cells and small organisms. This method permits the study of exogenous amyloid protein seeding endogenous protein present in normal cells. Our data indicate a greater pathological risk than hitherto suspected for extracellular soluble amyloid protein. Furthermore, our techniques offer new capabilities in the study of amyloid growth dynamics at the molecular level which will be important for the development of novel therapeutic strategies.

O-73 (P-469)**Interaction between amyloid oligomers and plasma membrane. A single cell force spectroscopy study**C. Canale¹, R. Oropesa-Nunez¹, S. Dante¹, C. Cecchi², M. Stefani², F. Chiti², A. Diaspro¹¹Nanophysics Department – Istituto Italiano di Tecnologia, Genova, Italy; ²Department of Biochemical Sciences – University of Florence, Florence, Italy

Cytotoxicity in amyloid diseases is mediated by small oligomeric species and, in particular, by their interaction with the plasma membrane. The level of toxicity depends from both the physicochemical properties of the protein aggregates and the composition and organization of the cell membrane.

We employed the small peptide HypF-N as a model of amyloidogenic peptide.

Considering these well demonstrated points:

1. HypF-N aggregates in different conditions, producing oligomers with different toxicity
2. cytotoxicity is always triggered by GM1 content in the cell membrane

We studied the interaction of the cell membrane with toxic and nontoxic protein misfolded oligomers by using AFM-based single cell force spectroscopy (SCFS). We quantified the affinity of the different oligomeric species for the lipid and protein fraction of the cell membrane. In particular, we observed, for the first time, that toxic oligomers influence the functionality of a large class of molecules involved in cell adhesion. We demonstrated that the ganglioside GM1 play a pivotal role in this mechanism.

Oral Presentations

– 12. Protein misfolding –

O-74 (P-470)

DNA PAINTing amyloid aggregates

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The aberrant misfolding and aggregation of soluble proteins into amyloid fibrils characterizes many neurodegenerative disorders, including Parkinson's and Alzheimer's disease. The ability to study such processes has remained difficult due to the heterogeneity and low abundance of the aggregates along the fibril-formation pathway. Many such species are smaller than the diffraction limit of light (~250 nm), and so imaging them in high enough resolution with optical microscopy has been limited. We report here a method, termed ADPAINT (aptamer DNA PAINT), for the characterization of amyloid species at the nanometer scale. Using a combination of DNA PAINT and an amyloid specific aptamer, we demonstrate that this technique is able to detect a whole range of aggregates species along the aggregation pathway of alpha-synuclein, allowing for the earliest formed oligomers as small as 50 nm to be imaged in high detail, both within cells and in the test tube.

O-75 (P-471)

Functional amyloids from the fungal pathogen *Aspergillus fumigatus*

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Hydrophobins are fungal proteins characterised by their amphipatic properties and an idiosyncratic pattern of eight cysteine residues. The soluble form of these proteins spontaneously self-associates at hydrophobic/hydrophilic interfaces to form **amphipatic layers with remarkable physico-chemical properties**. The **RodA** hydrophobin of the airborne fungal pathogen *Aspergillus fumigatus* forms a functional **amyloid layer** with rodlet morphology that covers the surface of the spores, rendering the latter hydrophobic and thus facilitating their dispersal. In addition, the **rodlet coat** masks **the spores**, which are the infectious morphotype, **from the immune system**. We have solved the solution structure of RodA, studied its self-assembly *in vitro*, performed a mutational analysis to highlight the regions involved in the formation of the amyloid core of the rodlets and on their lateral association to form layers, correlated the kinetics of rodlet formation *in vitro* with their rate of appearance on the spores and analysed the relationship between the structure of RodA and its immunological properties.

Oral Presentations**– 13. Mechanosensing and mechanoregulation –****O-76****Myosin-II filaments, microtubules, and cell-matrix adhesions: mechanical and signalling interactions**A. D. Bershadsky^{1,2}¹Mechanobiology Institute, National University of Singapore, Singapore; ²Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Although myosin II filaments are known to exist in non-muscle cells, their organization and dynamics are incompletely understood. Here, we combined structured illumination microscopy with pharmacological and genetic perturbations, to study the myosin II filaments self-organization, their regulation by microtubules, and their role in the regulation of integrin-mediated adhesions. A striking feature of the myosin II filaments organization was their alignment into superstructures (“stacks”), which required myosin-II contractility, as well as actin filament assembly-disassembly and crosslinking (dependent on formin Fmn13, cofilin1, and actinin-4). Disruption of microtubules or uncoupling them from integrin-based adhesions by knockdown of linker proteins KANK1 or KANK2 in macrophage-like cells and fibroblasts resulted in disassembly of podosomes, while promoted assembly of focal adhesions (FAs). Both these effects were accompanied by the assembly and self-organization of myosin II filaments. Knockdown of Rho GEF GEF-H1, or myosin-IIA, or suppression of myosin II filament assembly by inhibition of Rho kinase abolished microtubule-dependent regulation of both podosomes and FAs. Thus, microtubules control FAs and podosomes through GEF-H1-dependent reorganization of myosin II filaments.

O-78**Physical forces driving migration, division and folding of epithelial sheets**

X. Trepap

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Biological processes such as morphogenesis, tissue regeneration, and cancer invasion are driven by collective migration, division, and folding of epithelial tissues. Each of these functions is tightly regulated by mechanochemical networks and ultimately driven by physical forces. I will present maps of cell-cell and cell-extracellular (ECM) forces during cell migration and division in a variety of epithelial models, from the expanding MDCK cluster to the regenerating zebrafish epicardium. Force maps show that cells dividing in a migrating epithelium exert large cell-ECM forces during cytokinesis. These forces point towards the division axis and are exerted through paxillin-rich focal adhesions that connect the cytokinetic ring to the underlying ECM. Large forces at these adhesions are associated with failure of cytokinesis and polyploidy, indicating that abnormal cell-matrix adhesion at the cleavage furrow impedes abscission. Time lapse analysis of force maps further reveals that cell-cell forces determine the duration of the cell cycle and mitosis. Finally, I will present direct measurements of epithelial traction, tension, and luminal pressure in three-dimensional epithelia of controlled size and shape. Strikingly, we found that epithelial tension in the free-standing curved monolayers is constant up to 200% strain, indicating active mechanisms of tensional homeostasis.

O-77**Strain dependent mechanisms in regulation of striated muscle**

T. C. Irving

Illinois Institute of Technology, USA

The generally accepted sliding filament model of muscle contraction and the so-called steric blocking model of thin filament regulation does not explain all features of muscle contraction. Most muscles will generate more force at a longer sarcomere length, at the same level of activating calcium, than at the length where the muscle is slack. This phenomenon, ‘myofilament length dependent activation’, underlies the well-known Frank –Starling Law of the Heart. An understanding of the molecular mechanisms underlying myofilament length dependent activation has been elusive. Synchrotron small-angle X-ray diffraction is the ideal technique to obtain structural information on the sarcomeric structure of striated muscle in real physiological time I will review how muscle diffraction has provided evidence that myofilament length dependent activation in cardiac muscle has its basis in the transmission of strain generated by the giant protein titin when muscle is stretched causing structural changes in both the myosin –containing thick filaments and actin-containing thin filaments leading to enhanced activation. I will discuss how strain dependent structural changes in the myofilaments are also involved in the contraction of skeletal muscle as well as indicate some future prospects.

O-79 (P-498)**Topological defects in epithelia govern cell death and extrusion**A. Doostmohammadi¹, T. B. Saw², V. Nier³, L. Kocgozlu², S. P. Thampi⁴, Y. Toyama², P. Marcq³, C. T. Lim², J. M. Yeomans¹, B. Ladoux⁵¹University of Oxford, UK; ²National University of Singapore, Singapore; ³Sorbonne Universités, France; ⁴IIT Chennai, India; ⁵Université Paris Diderot, France

Epithelia remove excess cells through extrusion, and prevent accumulation of pathological cells. Despite the important links of cell extrusion to developmental and pathological processes such as cancer metastasis, its underlying mechanism is largely unexplored. Here, we show that apoptotic cell extrusion is provoked by singularities in cell alignments in the form of comet-like topological-defects. We find a universal correlation between the extrusion sites and positions of nematic defects in the cell orientation field in different epithelium types. We model the epithelium as an active nematic liquid-crystal and compare the numerical simulations to strain-rate and stress measurements within cell monolayers. The results confirm the active nematic nature of epithelia, and demonstrate that defect-induced isotropic stresses are the primary precursor of mechanotransductive responses in cells such as YAP transcription factor activity, caspase-3 mediated cell death, and extrusions. We further demonstrate the ability to control extrusion hotspots by geometrically inducing defects through microcontact-printing of patterned monolayers. Together we propose a novel mechanism for apoptotic cell extrusion: spontaneously formed topological defects in epithelia govern cell fate.

Oral Presentations**– 13. Mechanosensing and mechanoregulation –****O-80 (P-497)****Nanoscale mechanical modification in the brain tumour micro-environment**

E. Minelli¹, G. Ciasca¹, G. Campi², T. E. Sassun³, M. Antonelli³, M. Papi¹, A. Santoro³, F. Giangaspero³, R. Delfini³, M. de Spirito¹

¹Physics Institute, UCSC, Rome, Italy; ²IC-CNR, Monterotondo, Italy; ³Dept. of Neurology and Psychiatry and Dept. of Radiology, Oncology and Pathology, Sapienza, Rome, Italy

Glioblastoma multiforme (GBM) is an extremely aggressive brain tumour, characterized by a diffuse infiltration of neoplastic cells into the brain parenchyma. Although rarely considered, mechanical cues play a key role in the infiltration process, that is extensively mediated by the tumour microenvironment stiffness and by the occurrence of aberrant interactions between neoplastic cells and the extracellular matrix. In this work we provide the first nanomechanical characterization of the viscoelastic response of human GBM tissues by Atomic Force Microscopy. Our high resolution elasticity maps show a large difference between the biomechanics of GBM and the healthy peritumoral tissues and unveil the nanomechanical signature of necrotic regions and anomalous vasculature. Our findings open the way to the development of novel quantitative methods to assess the tumour grade, that can be used in combination with the conventional histological examinations. In order to provide a more in-depth description of the role of mechanical cues in tumour progression, we compared the nanomechanical fingerprint of GBM tissues with that of grade-I (WHO) meningiomas, a benign lesion characterized by a completely different growth pathway and a different biomechanical response.

O-81 (P-496)**Binding of ZO-1 to $\alpha 5 \beta 1$ regulates the mechanical properties of $\alpha 5 \beta 1$ -fn links**

V. González-Tarragó¹, A. Elósegui-Artola¹, E. Bazellères², R. Oria¹, C. Pérez-González¹, P. Roca-Cusachs^{1,3}

¹Institute for Bioengineering of Catalonia (IBEC), Spain;

²Institut de Biologie du Développement de Marseille (IBDM), France; ³University of Barcelona (UB), Spain

Fundamental processes in cell adhesion, motility, and rigidity adaptation are regulated by integrin-mediated adhesion to the extracellular matrix (ECM). The link between the ECM component fibronectin (fn) and integrin $\alpha 5 \beta 1$ forms a complex with ZO-1 in cells at the edge of migrating monolayers, regulating cell migration. However, how this complex affects the $\alpha 5 \beta 1$ -fn link is unknown. Here we show that the $\alpha 5 \beta 1$ /ZO-1 complex decreases the resistance to force of $\alpha 5 \beta 1$ -fn adhesions located at the edge of migrating cell monolayers, while also increasing $\alpha 5 \beta 1$ recruitment. Consistently with a molecular clutch model of adhesion, this effect of ZO-1 leads to a decrease in the density and intensity of adhesions in cells at the edge of migrating monolayers. Taken together, our results unveil a new mode of integrin regulation through modification of the mechanical properties of integrin-ECM links, which may be harnessed by cells to control adhesion and migration.

Oral Presentations**– 14. Correlative, multiscale and functional imaging –****O-82****Resolution passion**

P. Bianchini, G. Zanini, K. Korobchevskaya, M. Oneto, P. Luca, A. Diaspro
Nanoscopy, Istituto Italiano di Tecnologia, Genova, Italy, Italy

Since the invention of confocal microscopy and two-photon excitation microscopy and the advent of super-resolution microscopies, i.e. nanoscopy, the efforts in the development of novel optical microscopy techniques were mostly oriented to the improvement of the resolution power. Nowadays, this trend is still valid but opens to new flavours, e.g. application to living specimens and label free approaches(1). In particular, there are some novel techniques that does not necessarily involve fluorescence, e.g. polarization (2), subtraction (3), pump-probe (4) and expansion(5) microscopy. Those methods allow to overcome some of the limitations of actual super-resolved microscopy, enabling applications that were not accessible before. Nevertheless, fluorescence remains a key player, it validates in a correlative approach that a novel technique returns reliable results. During my talk I will show some novel experiments exploiting pump-probe and expansion nanoscopy techniques respectively, realized in a STED fashion way.

1. Bianchini, P., et al. 2008 J. Biophoton. 1, 443–450.

2. Mazumder, N., et al. 2017 J. Opt., 19, 025301.

3. Korobchevskaya, K., et al. 2016 Sci. Rep. 1-9.

4. Liu, N., et al. 2016 ACS Photonics. 3: 478–485.

5. Chen, F., et al. 2015. Science. 347: 543–548.

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O-84**Integrating 3D light and electron microscopy for multiscale correlative imaging**

L. M. Collinson
Francis Crick Institute, UK

Correlative light and electron microscopy (CLEM) combines the benefits of fluorescence and electron imaging, revealing protein localisation against the backdrop of cellular ultrastructure.

To image rare events in cells, tissues and whole model organisms, we developed Correlative Light and Volume EM (3D CLEM), which combines correlative workflows with microscopes that automatically collect large stacks of high resolution images.

To increase protein localisation precision, we developed an ‘In-Resin Fluorescence’ (IRF) protocol that preserves the activity of GFP and related fluorophores in resin-embedded cells and tissues. The sample preparation is relatively fast, and also introduces electron contrast so that cell structure can be visualised in the electron microscope. Once the resin blocks have been cut into ultrathin sections, out-of-plane fluorescence is removed resulting in physical ‘super-resolution’ light microscopy in the axial direction, which increases the accuracy of the LM-EM overlays. Localisation precision is further increased by imaging the IRF sections *in vacuo* in the next generation of commercial integrated light and electron microscopes (ILEM). We further improved accuracy by developing integrated super-resolution light and electron microscopy.

O-83**Correlation of 3D structure and chemical determination at whole cell level by near-edge soft X-rays nanotomography**

J. L. Carrascosa
Centro Nacional de Biotecnología, Department of Structure of Macromolecules. Calle Darwin 3; 28049 Madrid, Spain

Soft X-ray microscopy (SXM) is a relatively new approach which provides great penetration and natural contrast on biological samples in the water window (284 eV to 543 eV), thus allowing to obtain tomographic reconstructions of whole cells in near-native state using fast freezing sample preparation (Schneider et al., 2010; Chichon et al., 2012). To test the suitability of SXM to retrieve quantitative information, we have studied the interaction of super-paramagnetic iron oxide nanoparticles (SPIONs) with a breast cancer cell line. Fluorescent microscopy and Transmission electron microscopy were correlated to define the different steps of the process. Then, whole tomograms obtained by cryo-SXM were studied to retrieve 3D data for quantitative statistical analysis of the intracellular SPION accumulation (Chiappi et al., 2016). Finally, the 3D structural map was merged with the quantitative determination of the iron oxide nanoparticle content inside cells, which was obtained using near-edge absorption soft X-ray nanotomography (Conesa et al., 2016), exploiting the iron oxide differential absorption contrast at specific X-ray energies. Our results demonstrate the potential of SXM for providing structural information and elemental quantitative determination at 50 nm resolution in 3D.

O-85 (P-517)**Using STORMForce for understanding how bacteria grow and die**

R. K. Tank, R. D. Turner, N. Mullin, C. Ashley, F. J. Simon, H. K. Jamie
The University of Sheffield, UK

AFM is able to achieve nanometre resolution, and to sensitively measure forces to study molecular and cellular processes. However AFM is only able to do this on the surface of a sample, where STORM uses fluorescent labels to carry out sub-optical diffraction limit imaging. This enables the user to label specific proteins within the membrane whilst force mapping or imaging the environment that it is in. Here we studied the cell wall of *E. coli* bacteria. The details of the architecture and the way in which that architecture is formed are still poorly understood.

Firstly a STORMForce image was obtained through imaging on separate equipment and overlaying the images. A protocol has been developed that is suitable for both AFM and STORM.

For an integrated STORMForce image an AFM image was taken 1st and then a STORM image was obtained from the same area. Progress towards molecular resolution AFM using “Hyperdrive” with molecular localisation using STORM on the combined STORMForce instrument will be presented.

Oral Presentations**– 14. Correlative, multiscale and functional imaging –****O-86 (P-518)****Study on acoustic signal features influenced by thermoacoustic effects in magnetoacoustic tomography**

X. Zhou, J. Li, S. Liu, S. Zhang, T. Yin, Z. Liu

Chinese Academy of Medical Sciences & Peking Union Medical College, China

Magnetoacoustic tomography (MAT) is an emerging non-invasive electrical conductivity imaging method that combines the high dielectric contrast of tissues and excellent resolutions of ultrasonography, which is a promising modality for tumor early diagnose. In our study, we have found that the thermoacoustic (TA) effect occurred in coincidence with the measurement of acoustic signal in MAT. In order to investigate the influence of the TA effect on the acoustic signal, experiments on several materials with different conductivities were conducted by a MAT system both with and without a static magnetic field. The acoustic signals were analyzed then compared in the time domain and the frequency domain, respectively. The TA effect is found to be related to material characteristics. For tissue-like materials with low conductivities, the TA signals caused by the TA effect are observable and cannot be ignored in both domains. This demonstrates that the TA effect occurred in MAT would influence the acoustic signal features. More rigorous algorithms should be developed for the MAT system in the future, to obtain better imaging quality.

O-87 (P-519)**Cell-temperature mapping by Eu-doped TiO₂ nanothermometers**M. Garvas¹, B. Kokot¹, I. Urbancic¹, R. Podlipec¹, Z. Arsov¹, P. Umek¹, C. Bittencourt², J. Strancar¹, T. Koklič¹¹J. Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia; ²Université de Mons, Parc Initialis, Avenue N. Copernic, 1, B-7000 Mons, Belgium

Temperature regulates many biomolecular reactions within cells. Measuring of temperature on a nanometer scale has therefore presented a desirable challenge for the last two decades. Several biocompatible nanothermometers have been proposed as potential sensors, including lanthanide nanoparticles, quantum dots, gold nanoparticles, fluorescent polymers, and polymers with encapsulated fluorophores. To overcome the problem related to possible temperature gradients in a cell, precise nano-scale temperature measurements has been done also in this work using Eu³⁺-doped TiO₂ nanospheres. This material is known to adhere to membranes and can even be internalized. Our experiments clearly show that a temperature increase of few K can be easily detected. Temperature measurement with the Eu³⁺-doped TiO₂ nanospheres should be artifact-free, since Eu³⁺ atom is incorporated in a TiO₂ lattice, and thus isolated from intracellular environment. On the other hand, the sensor is still very small, with a size of few nanometers only enabling to detect local temperature gradients if they exists.

Oral Presentations**– 15. Evolution, ecology, collective and emergent behaviour –****O-88****Tolerance and persistence promote the evolution of antibiotic resistance**

I. Levin-Reisman, O. Gefen, N. Q. Balaban

The Hebrew University, Physics Department, Jerusalem, Israel

Controlled experimental evolution during antibiotic treatment can shed light on the processes leading to antibiotic resistance in bacteria. Recently, intermittent antibiotic exposures have been shown to lead rapidly to the evolution of tolerance, i.e. the ability to survive under bactericidal treatments without developing resistance. However, whether tolerance delays or promotes the eventual emergence of resistance is unclear. We used *in vitro* evolution experiments to explore this question. We found that in all cases tolerance or persistence, i.e. heterogeneous tolerance, preceded resistance. A mathematical population-genetics model showed how tolerance boosts the chances for resistance mutations to spread in the population. Thus, tolerance mutations pave the way for the rapid subsequent evolution of resistance. Preventing the evolution of tolerance may offer a new strategy for delaying the emergence of resistance.

O-90**Gene regulation from an evolutionary perspective**O. Rivoire¹, I. Junier²¹CIRB, Collège de France & CNRS, France; ²TIMC-IMAG, Université Grenoble Alpes & CNRS, France

Transcriptional regulation in bacteria is often pictured as a network of operons controlled by transcription factors. This architecture, however, accounts only for part of the coordination of gene expression. I'll present an alternative picture derived from an evolutionary analysis of hundreds of bacterial genomes which suggests that the basic units of regulation are not necessarily operons and that their control does not necessarily involve transcription factors.

O-89**Evolutionary dynamics in the polarity network in budding yeast**W. K.-G. Daalman¹, E. T. Diepeveen¹, F. Brauns², E. Frey², L. Laan¹¹Bionanoscience Department, Delft University of Technology, Delft, Netherlands; ²Arnold Sommerfeld Center for Theoretical Physics (ASC), Ludwig-Maximilians-Universität München, Germany

Cells are organized by functional networks, which typically contain components whose removal severely compromises the network's function. Despite their importance, these components are not well conserved between the tree of life: using bioinformatics we compared the conservation of 34 polarization genes from budding yeast between 200 different fungal strains and species and found that the vast majority of species contains a unique combination of polarity genes. This diversity suggests that cells can evolve to perform a similar biological functions with different proteins.

To study network evolution we evolved *Saccharomyces cerevisiae* for 1000 generations without an important polarity gene. At the end of the evolution the lineages had dramatically increased in fitness. Sequencing their genomes and monitoring polarization revealed a common evolutionary trajectory, with a fixed sequence of adaptive mutations, each improving cell polarization by inactivating proteins. We now combine kinetic modelling and live-cell microscopy to obtain a quantitative molecular understanding. Our goal is to explain how proteins that are essential in one species can be absent in a closely related species, while both species have a similar functional requirement.

O-91 (P-529)**Dynamics of bacterial community architecture governs viral protection and dispersal mechanisms**K. Drescher^{1,2}¹Max Planck Institute for Terrestrial Microbiology, Germany; ²Department of Physics, Philipps University Marburg, Germany

In nature, bacteria primarily live in communities, termed biofilms that have many emergent properties. In medical settings, biofilms cause devastating damage during infections; indeed, bacteria are often viewed as agents of human disease. However, bacteria themselves suffer from diseases, mostly in the form of viral pathogens termed bacteriophages, which are the most abundant replicating entities on Earth. Phage-biofilm encounters are extremely common in the environment, but the mechanisms that govern these interactions are unknown. Using *E. coli* biofilms and the lytic phage T7 as models, we discovered that an amyloid fiber network protects biofilms against phage attack *via* two separate, novel mechanisms. First, collective cell protection results from inhibition of phage transport into the biofilm. Second, amyloid fibers protect cells individually by coating their surface and binding phage particles, thereby preventing their attachment to the cell exterior. We also discovered that the matrix dynamics governs the dispersal process of cells from biofilms. These insights into biofilm-phage interactions and matrix architecture have broad-ranging implications for phage applications in biotechnology, phage therapy, and the evolutionary dynamics of phages with their bacterial hosts.

Oral Presentations

– 15. Evolution, ecology, collective and emergent behaviour –

O-92 (P-530)**Stochasticity and division of labour in toxin production in two-strain bacterial competition in *E.coli***

B. von Bronk, S. A. Schaffer, A. Götz, M. Opitz

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Interactions within bacterial communities play a crucial role in community composition dynamics and maintenance of biodiversity. Here we show that competition of an *E.coli* strain C, which performs division of labor between reproducers and self-sacrificing toxin producers, with a toxin sensitive strain S, can lead to four different outcomes: dominance of S or C, coexistence, and extinction of both strains. To disentangle the impact of division of labor and stochasticity in toxin production on competition and C strain success, we employed an experimental approach that permits observations of the competition at multiple length-scales (near single cell to macroscopic levels). We found that the stochastic toxin production dynamics affect the competition twofold. First, in the initial phase ($t < 12\text{h}$), it influences the formation of viable C clusters at the colony edge. Second, it determines the toxin producer fraction within the C population, which dictates the deterministic competition dynamics in the second phase. In addition, we developed a stochastic and spatially extended computational model of the competition to complement the experiments. Our findings enhance the understanding of division of labor and the importance of stochasticity in toxin production for bacterial competition.

O-93 (P-531)**Collective feeding in *C. elegans***L. J. Schumacher¹, S. Ding², A. E. X. Brown², R. G. Endres¹¹Life Sciences, Imperial College London, UK; ²MRC London Institute of Medical Sciences, Imperial College London, UK

Collective behaviour is frequently studied in groups of large animals but is less well understood in small organisms and at mesoscopic scales. Here we investigate the collective feeding behaviour of the nematode worm *C. elegans*, well known for its easy genetic manipulability and stereotypic, yet complex behaviour.

By tracking many worms and probing the dynamics inside aggregates with fluorescent imaging we quantify behavioural differences between the “asocial” lab strain, “social” wild isolates, and an aggregating mutant strain.

Drawing on concepts from motility-induced phase transitions in active matter and bacterial systems, we interrogate the mechanism of aggregation by quantitative analysis of tracking data and computational modelling of worm movement. Our study elucidates how small genetic differences affect the emergent phenotypes of group behaviour, and how social information flows from individuals to the population.

Oral Presentations

– 16. Molecular and cellular processes of energy transduction –

O-94

Efficient energy transduction in respiratory complexes and supercomplexes

W.-C. Kao¹, C. Wirth¹, V. Pandey^{1,2}, C. Hunte¹¹Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany; ²Faculty of Biology, University of Freiburg, Freiburg, Germany

Fascinating cofactor-bearing multi-subunit membrane protein machineries enable biological energy conversion in all kingdoms of life. Cofactor-mediated transfer of electrons requires fine-tuned redox potentials and precise spatial and temporal control of distance, orientation and environment of cofactors. By-pass reactions in the mitochondrial respiratory complexes I and III can lower the efficiency of the bioenergetic machines and generate reactive oxygen species (ROS) which are harmful at elevated concentration. Mechanistic and structural features such as the A/D transition in complex I (1) and the Qo motif in complex III (2) control by-pass reactions. Respiratory supercomplexes may fulfill a similar function. We characterized an interesting model system, the cytochrome *bcc-aa₃* supercomplex from the Actinobacterium *Corynebacterium glutamicum*, closely related to *C. diphtheriae* and *Mycobacterium tuberculosis*. We had predicted low redox potentials for the cyt *bcc* complex on the basis of the PDWY sequence for the Qo motif of actinobacterial cyt *b* (2), which are now experimentally confirmed. Redox midpoint potentials are fine-tuned permitting efficiently coupled menaquinol oxidation and reduction of dioxygen in one molecular entity, in an obligate supercomplex (3).

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O-96 (P-551)

Coupling fluorescence microscopy and electrochemistry to investigate single mitochondria metabolism

V. S. Vajrала¹, F. Sekli Belaïdi², G. Lemerrier², D. Zigan¹, J. Launay², P. Temple-Boyer², N. Sojic¹, S. Arbault¹¹University of Bordeaux, CNRS, INP-Bordeaux, France;²LAAS-CNRS, Université de Toulouse, France

Mitochondria are major cell organelles as being the main source of ATP through the oxidative phosphorylation. They also play major roles into other metabolic pathways (Krebs cycle, lipid synthesis, calcium, redox signalling) and when defective, they are involved into severe pathologies (myopathies, neurological disorders...). Consequently, new methodological approaches are required to decipher mitochondrial activities and provide tools for diagnosis. We have developed microsystems, ElecWell platforms, which combine electrochemical and optical sensing abilities. These are based on the integration of platinum nanoelectrodes (RNE, 200 nm thickness) into SiO₂-based microwell arrays (10² to 10⁶ wells; vol. < 1 pL). RNE exhibit high current density, fast response time, and high S/N ratio. The glass substrate of microsystems allows the observation by microscopy within all wells. A suspension of mitochondria is let to sediment on the array; mitochondrial trapping in each well is monitored by fluorescence, owing to their NADH or membrane potential. Simultaneously, we monitor electrochemically their oxygen consumption rate in response to activators and inhibitors of the respiratory chain. ElecWells offer unprecedented resolution to assess single mitochondria activities and dysfunctions.

O-95

Structure of complete ATP synthase and its role as new drug target against tuberculosis

T. Meier

Imperial College London, Department of Life Sciences, UK

F₁F_o-ATP synthases are paradigmatic molecular machines, which use the transmembrane electrochemical ion gradient to power ATP synthesis. The enzymes belong to the class of rotary ATPases, which all share a common architecture principle, consisting of a rotor and stator entity. While ions are shuttled through the F_o complex of the enzyme, torque is generated at the rotor/stator and transferred to the F₁-catalytic subunits for ATP synthesis. In the opposite direction, ATP hydrolysis can be used to drive ion pumping. In my talk I will present the structure of complete ATP synthase analysed by X-ray crystallography and cryo-electron microscopy. I will also focus on biochemical and structural investigations of the ATP synthase with respect to the development of new antibiotics in the fight against infectious diseases such as tuberculosis.

O-97 (P-552)

Retinal thermal equilibrium, photocycle and energy conversion in the microbial seven-transmembrane photoreceptors

X.-Y. Ding^{1,2}, C. Sun¹, H.-L. Cui¹, Y.-J. Gao¹, J. Wang¹, Y.-N. Yang¹, A. Watts³, X. Zhao¹¹East China Normal University, China; ²Penn State College of Medicine, USA; ³University of Oxford, UK

Light-driven proton pumps, a number of the microbial rhodopsin family seven-transmembrane receptors, distribute widely among Archaea, Eubacteria, and Eukaryota for harvesting and converting light energy in a wider spectral range. The common feature of those proteins is that the retinal chromophore maintains a *cis-trans* thermal equilibrium in the dark-adapted state. Absorption of a photon causes photoisomerization of the chromophore from the all-*trans* to the 13-*cis*, 15-*anti* configuration that triggers a series of structural rearrangements in the protein and initiates the vectorial translocation of a proton out of the cell. However, little is to know about how the retinal *cis-trans* thermal equilibrium affects the proton translocation mechanism and further impacts on the energy conversion rate. 2D solid-state NMR of specifically labelled receptors, reinforced with molecular dynamic simulations, mutational analysis and functional assays, supported by and compared with rigid-atom crystal structural models are employed to address this challenge. Shifting of the retinal *cis-trans* thermal equilibrium to an either *cis* or *trans* dominated state will affect the proton photocycle mechanism and further decrease the energy conversion rate.

Oral Presentations

– 16. Molecular and cellular processes of energy transduction –

O-98 (P-553)**Protons at the membrane water interface**E. Weichselbaum, D. G. Knyazev, P. PohlInstitute of Biophysics, Johannes Kepler University Linz,
4040 Linz, Austria

Proton diffusion along biological membranes is vitally important for cellular energetics. It occurs almost as fast as that in pure water (Serowy *et al.* 2003. *Biophys. J* 84:1031). Protons migrate without significant contributions from jumps between ionizable groups on the membrane surface (Springer *et al.* 2011. *PNAS* 108:14461). The process requires nothing but structured water at the boundary of a hydrophobic phase (Zhang *et al.* 2012 *PNAS* 109:9744). So far the origin of the Gibbs activation energy barrier ΔG^\ddagger_r remained enigmatic that opposes proton surface-to-bulk release. Here we determined ΔG^\ddagger_r from Arrhenius plots of (i) protons' surface diffusion constant and (ii) surface-to-bulk release rates. We found both parameters by (α) photo-releasing protons from a membrane patch at different temperatures and (β) monitoring their arrival at a distant patch. The results disproved that quasi-equilibrium exists between protons in the near-membrane layers and in the aqueous bulk. Instead, non-equilibrium kinetics is consistent with this experiment. ΔG^\ddagger_r only contains a minor enthalpic contribution that roughly corresponds to the breakage of a single hydrogen bond. This work reconciles the delayed proton surface-to-bulk release with protons weak bonding to surface water molecules.

Oral Presentations**– 17. Membrane permeation: channels –****O-99****Single-molecule spectroscopy of folding and assembly of the cytolytic pore toxin ClyA**

B. Schuler

University of Zurich, Switzerland

Pore-forming toxins are protein assemblies utilized by many organisms to disrupt the membranes of target cells. They are expressed as soluble monomers that assemble spontaneously into multimeric pores. In the case of the bacterial cytolytic toxin ClyA, this process involves a remarkably large conformational rearrangement in the protein. To investigate the implications of this conformational bistability and the assembly mechanism of the resulting ring-shaped, homodecameric pores, we used a combination of kinetic data from single-molecule spectroscopy and complementary techniques on timescales from milliseconds to hours, and from picomolar to micromolar ClyA concentrations. The entire range of experimental results can be explained quantitatively by a surprisingly simple assembly mechanism. First, addition of the detergent n-dodecyl- β -D-maltopyranoside to the soluble monomers triggers the formation of assembly-competent toxin subunits, accompanied by the transient formation of a molten-globule-like intermediate. Then, all sterically compatible oligomers contribute to assembly, which greatly enhances the efficiency of pore formation compared to simple monomer addition. However, during folding of ClyA in the absence of detergent, a remarkably persistent misfolded helical state is formed.

O-101**Direct pharmacological targeting of a mitochondrial ion channel eliminates cancer cells *in vivo***L. Leanza¹, M. Azzolini², L. Biasutto², F. Severin³, V. Martini³, L. Trentin³, A. Mattarei⁴, M. Zoratti², C. Paradisi⁴, E. Gulbins⁵, I. Szabo¹¹Department of Biology, University of Padova, Italy; ²CNR Institute of Neurosciences, University of Padova, Italy;³Department of Medicine, University of Padova, Italy;⁴Department of Chemistry, University of Padova, Italy;⁵Department of Molecular Biology, University of Duisburg-Essen, Germany

Ion channels are emerging oncological targets as their modulation affects proliferation as well as apoptosis. On the other hand, mitochondria are also important oncological targets due to their crucial role in apoptosis. Recently, we identified a therapeutic tool that simultaneously exploits both the high expression of the potassium channel Kv1.3 in the mitochondria of various types of cancer cells and the characteristic altered redox state of malignant cells, thereby leading to the selective elimination of even chemoresistant malignant cells by two novel mitochondria-targeted Kv1.3 inhibitors. Importantly, the strong tumor-reducing effects were not accompanied by immune-depression, cardiac toxicity or histological alteration of healthy tissues. These inhibitors also killed 98% of *ex vivo* primary chronic B-lymphocytic leukemia tumor cells, while sparing healthy B cells from the same patients and preliminary data suggest efficacy of the drug also in an animal model of B-CLL. These findings thus offer the perspective of a major advance in the pharmacological treatment of some high-impact, poor-prognosis cancers by modulation of ion channel activity.

O-100**Roles of Aquaporins in health and disease: promising targets for drug discovery**A. F. Mósca¹, A. de Almeida², C. Rodrigues¹, A. Casini², G. Soveral¹¹Faculdade de Farmacia, Universidade de Lisboa, Portugal;²School of Chemistry, Cardiff University, UK

Aquaporins (AQPs) are membrane channels involved in the transport of water and glycerol, crucial for many biological functions such as transepithelial fluid transport, cell migration and proliferation, brain water balance and adipocyte metabolism. AQPs are also involved in tumor invasion, metastasis and spread. In the last decade, the aquaporin field became a very hot area of research with increasing physiological and medical implications.

We have reported the AQP3 inhibition properties of a number of gold-based compounds by stopped-flow spectroscopy. *In vitro* studies, also taking advantage of our unique inhibitors, have highlighted the relation between AQP3 expression, glycerol permeation across cell membranes and cell proliferation. Additional studies focusing on different ligand scaffolds established preliminary structure-activity relationships, and confirmed the necessity of gold ions to achieve AQP inhibition. The mechanism of inhibition has been recently described using molecular dynamics, combined with density functional theory and electrochemical studies.

The high selectivity and potency of gold compounds makes them suitable drug leads for *in vivo* studies and points to aquaporin modulators as novel therapeutic and diagnostic agents.

O-102 (P-568)**Mechanism of loop C closure in the glycine receptor and its relevance for partial agonism**M. A. Dämgen¹, T. Greiner², R. Lape², L. G. Sivilotti², P. C. Biggin¹¹Structural Bioinformatics and Computational Biochemistry, Department of Biochemistry, University of Oxford, UK;²Department of Neuroscience, Physiology and Pharmacology, University College London, UK

Cys-loop receptors mediate fast synaptic transmission and are pivotal drug targets. Agonist binding opens the transmembrane pore, allowing ions to flow into the cell. Previous research has identified agonists with varying ability to open the ion channel, yet an explanation of partial agonism at atomistic resolution remains elusive. An understanding would be crucial for drug design, since depending on the clinical situation, the ideal therapeutic drug should elicit a fine-tuned ion flow that is somewhere in between that of a full agonist and a silent antagonist. We performed molecular dynamics simulations of the glycine receptor with the full and partial agonists in the orthosteric binding site. For the first time, we report a detailed atomistic mechanism of loop C closure, which is the first step in the signal-transduction mechanism. Our findings suggest that agonist efficacy is linked to the ability of stabilising loop C in a closed conformation. Moreover, we find that a stable water molecule in the binding pocket plays a crucial role for all examined ligands. The observed glycine binding mode is in excellent agreement with a recent crystal structure where density of glycine with a stable water molecule in the binding pocket is discernible.

Oral Presentations**– 17. Membrane permeation: channels –****O-103 (P-570)****The Structure of an Open Activated Sodium Channel Reveals the Molecular Basis of Gating and Disease**

A. Sula, J. Booker, B. A. Wallace

Birkbeck College, University of London, UK

Voltage-gated sodium channels (Navs) play essential roles in excitable tissues, with the activation and opening of these channels resulting in the initial phase of the action potential. The cycling of Navs through closed and inactivated states and the related opening/ closing of other ion channels lead to exquisite control of intracellular ion concentrations in both prokaryotes and eukaryotes.

Our new high resolution crystal structure of the NavMs prokaryotic sodium channel (Sula et al, 2017), provides the only view to date of an open activated channel. It shows the interactions of the voltage sensor, S4-S5 linker, pore, and C-terminal domains (CTDs). The S4 helix is in an activated conformation and the pore gate is open at the intracellular surface. The CTD is coiled-coil that acts as a “glue” between the monomers that comprise the tetrameric channel. The conformation includes a heretofore unseen extensively hydrogen-bonded and salt-bridged interaction motif involving the sodium-channel specific S3 Trp, the S4-S5 linker, the end of the S6 transmembrane region and the top of the CTD. This structure provides the basis for understanding the processes of gating and ion translocation in Navs, and a novel insight into one of the basis of human diseases.

O-104 (P-571)**The mechanism of drilling β -barrel pores into lipid membranes by an earthworm protein Lysenin**

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Pore-forming proteins (PFPs) are widespread in nature and have important physiological roles in attack and defense mechanisms via formation of pores in lipid membranes of target cells. They are classified as either α - or β -PFPs, based on the secondary structure elements that form the transmembrane part of the pore. Members of aerolysin family form β -barrel shaped pores. The family expands from bacteria to vertebrates, with many bacterial representatives serving as crucial virulence factors. Lysenin is one of the few described eukaryotic members of this family from the earthworm *Eisenia fetida*. It is present in the coelomic fluid of earthworms to act defensively against parasitic microorganisms. It has a high affinity for sphingomyelin in membranes, and can be used as a tool for visualizing distribution and dynamics of sphingomyelin in cells. I will present the crystal structure of the lysenin pore (Podobnik et al., *Nat Comm* 2016), which provides important insights into the mechanism of pore assembly as well as general features of this family of nanopores. Our results are relevant for understanding of pore formation by other aerolysin-like PFPs involved in bacterial pathogenesis, as well as potential application of such pores in medicine and nanobiotechnology.

Oral Presentations**– 18. Modelling, inference, big data –****O-105****Software scalability and validation in big data analysis**J. W. K. Ho^{1,2}¹Victor Chang Cardiac Research Institute, Australia; ²St. Vincent's Clinical School, UNSW Sydney, Australia

This talk examines two important aspects of modern big data analysis – software scalability and validation. Scalability has always been an important consideration when developing analysis algorithms and programs. Nonetheless the surge of volume and variety of biological and biomedical data has posed new challenges. I discuss the historical development of various parallel and distributed computing technologies, and discuss how modern cloud computing paradigm is being used to deal with the scalability issue associated with big biological data analyses. Validation of the correctness of software output is another important issue in big data analysis. In particular, I survey the challenges associated with determining the correctness of the computational output of big data analysis software, and discuss how state-of-the-art software testing techniques, such as metamorphic testing, can be used to implement an effective quality assurance strategy. I hope this talk raises awareness of these critical issues in modern big data analysis.

O-107 (P-610)**Neuronal signaling pathways estimated from whole-brain imaging data of *C. elegans***Y. Iwasaki¹, T. Teramoto², S. Oe², T. Tokunaga³, O. Hirose⁴, S. Wu⁵, Y. Toyoshima⁶, M. S. Jang⁶, R. Yoshida⁵, Y. Iino⁶, T. Ishihara²¹Ibaraki University, Japan; ²Kyushu University, Japan; ³Kyushu Institute Technology, Japan; ⁴Kanazawa University, Japan; ⁵Institute of Statistical Mathematics, Japan; ⁶University of Tokyo, Japan

The nematode *C. elegans* is a useful model organism in neurobiology because the synaptic connectivity between all the 302 neurons has been completely identified. Recently, we developed a high-speed 4D calcium imaging system to measure neuronal activities in the whole central nervous system of *C. elegans*. We estimate neuronal signaling pathways in the whole central nervous system from the imaging data. In *C. elegans*, the result is able to be compared with the identified synaptic pathway. Neuronal signaling pathway is determined by the correlation coefficient and the Granger causality test. The correlation coefficient is commonly used to quantify undirected influence between two data. On the other hand, the Granger causality test quantifies directed influence ("predictive causality"). We find that actually used signaling pathways are different from the synaptic pathways in *C. elegans*. Some causalities between two neurons are derived from detour routing via the third neuron but not via two or more neurons. Causality network consists of the synaptic pathways within the second nearest neighbor distance. The transfer entropy, which is another causality detection based on information theory, is also discussed.

O-106**From single cells to populations: statistical models for heterogeneous biological systems**

C. Yau

University of Birmingham, UK

High-throughput single cell technologies have radically altered our ability to interrogate heterogeneous biological systems allowing us to measure differences in cellular behavior over space and time. The data produced by these new technologies allow novel scientific questions to be asked but also present many new analytical challenges. In this talk, I will present my recent research into modeling large-scale single cell 'omics data sets. I will discuss the fundamental statistical obstacles in developing algorithmic methods that scale to the high dimensionality and sample sizes of modern single cell data sets and the challenges of integrating with classical population data. My talk will be focused on our efforts to use single cell techniques in cancer research where we are simultaneously developing new analytical techniques alongside experimental methods. Finally, I shall also describe how our single cell analytical techniques are also leading to novel applications in other large data settings.

O-108 (P-609)**Colonization dynamics of bacteria in mice**F. Bansept¹, E. Slack², R. R. Regoes³, R. Voituriez¹, C. Loverdo¹¹Laboratoire Jean Perrin, UPMC-CNRS, France; ²Institute of Microbiology, ETH Zürich, Switzerland; ³Institute of Integrative biology, ETH Zürich, Switzerland

Our work aims at developing a stochastic model for the colonization of an organism by bacteria, applied to experimental data on mice gut colonization by the salmonella. We work both analytically and by simulations to extract biologically relevant parameters – like growth and death rates – from the indirect experimental data, which consist of the final proportions of genetically tagged bacteria. For some experiments, the results obtained using different observables characterizing the tags distribution are not compatible. We tested a model with two subpopulations of bacteria growing at different rates and showed that it allows to get qualitatively closer to the experimental observables, even if it is still quantitatively insufficient.

In experiments on the effects of a vaccination on this system, while the total number of bacteria is similar, the tag distribution is more variable in the case of previous vaccination or when immunoglobulin A is given to the mice, than in the absence of such a treatment. When immunoglobulin A is present, daughter bacteria remain stuck together after division. This leads to a smaller effective bacterial population size. When implementing this in the model, we could show that it could indeed explain the experimental tag distribution.

Oral Presentations

– 18. Modelling, inference, big data –

O-109 (P-608)**Transforming protein sequence and composition into numbers: A BIG DATA analysis tool for proteomes**

S. Prasad, R. Swaminathan

Indian Institute of Technology Guwahati, Assam, India

Protein sequences represented as strings of alphabetical characters are poorly suited for BIG DATA analysis of the vast proteome information. Further, the physicochemical properties of amino acids are lost by such representation. Here we present a novel approach, addressing the twin issues above by coding each natural amino acid in protein sequence as a prime number. The magnitude of assigned number was based on the hierarchy of the amino acid in the hydrophobicity scale. For example, most polar arginine was 2, while least polar isoleucine was 1831. As prime factors of any integer are unique, the inherent advantages of this numerical transformation are manifold, namely: a) Amino acid composition of any sequence can be stored as prime product (ProtID) of all residues in sequence; b) Base 2 logarithm of ProtID yields PS-Score that can serve as an identity tag of protein sequence composition, besides providing ready information on average residue polarity in the sequence; c) Coarse grain representation of protein sequence in a contiguous block of 3/5/10 residues is possible as local ProtIDs; d) Finally all proteins in a given proteome can be sorted based on their PS-Score values. Insights gained from analysis of proteomes ranging from bacteria to humans, shall be presented.

Oral Presentations

– 19. Experimental and computational approaches to protein design –

O-110**Minimalist design of protein and peptide catalysts**

I. V. Korendovych
Syracuse University, USA

Design of a novel catalytic function in proteins and peptides, apart from its inherent practical value, is important for fundamental understanding of the principles that govern enzymatic activity. Two representative cases applications of a computationally inexpensive, minimalistic approach to design of artificial enzymes will be presented.

1. Single amino acid residue mutations in a 74-residue-long C-terminal domain of calmodulin confer catalytic activities (Kemp elimination, ester hydrolysis, retroaldol reaction) onto this non-enzymatic protein. The catalytic efficiencies of the resulting allosterically regulated catalysts are on par with those of the best computational approaches. Directed evolution allowed for further improvement of catalysts' efficiency. 2. We designed a series of 7-residue peptides that self-assemble into amyloid-like fibrils to act as zinc-dependent esterases and copper-dependent monooxygenases with high catalytic efficiencies. These results indicate that amyloid fibrils are able to not only catalyze their own formation – they also can catalyze chemical reactions. This work has implications for the design of self-assembling nanostructured catalysts including ones containing a variety of biological and non-biological metal ions.

O-112**Advancing metalloprotein design for non-biological function**

A. F. Peacock, L. N. Slope, M. R. Berwick, S. L. Newton, A. Sellamuthu, O. J. Daubney, M. M. Britton
University of Birmingham, UK

De novo designed miniature protein scaffolds, such as the coiled coil, offer exciting opportunities for metal ion coordination.[1] Not surprisingly due to the protein like nature of the scaffold, the large majority of *de novo* metallocoiled coil examples have focussed their efforts on mimicking the active sites of native metalloenzymes. Our approach is to instead to use these artificial proteins as novel ligands for the coordination of xeno metals, with no known biological role, with the view to developing functional systems for valuable applications beyond the scope of nature. Herein, we focus on the design of lanthanide coiled coils for potential applications in MRI.[2,3] We report the redesign of these lanthanide binding sites and describe how various design variables impact on the resulting lanthanide coordination chemistry, and their subsequent efficiency as MRI contrast agents. Generous financial support from The University of Birmingham, the School of Chemistry and EU COST action CM1105 is acknowledged.

References

1. A. F. A. Peacock, Cur. Opin. Chem. Biol. 17 (2013) 934-939.
2. M. R. Berwick *et al.*, J. Am. Chem. Soc. 136 (2014) 1166-1169.
3. M. R. Berwick *et al.*, Chem. Sci. 7 (2016) 2207-2216.

O-111**Reaching the Pareto frontier in multi-objective protein design**

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¹CABM and Department of Biochemistry and Molecular Biology, Rutgers University, USA; ²Department of Computer Science, Tel Hai University, Israel

Traditional simulation-based protein design considers energy minimization of candidate conformations as a single-objective optimization problem. However, many design problems have more than one objective – i.e. protein stability, biocompatibility, immunogenicity, target affinity, pharmacokinetics, cell permeability, and so forth. Addressing multi-objective design problems requires new computational methods. The goal is to reach a condition known as Pareto Optimality where no single objective can be further improved without deteriorating other objectives. We consider a two-objective combinatorial minimization problem based on concurrent optimization of stability and specificity of heteromeric collagen mimetic peptides. The ability to test predictions of such calculations in the laboratory provides a useful benchmark for comparing evolutionary and non-evolutionary algorithms currently employed in the field.

O-113 (P-618)**Designing artificial TIM-barrel proteins from scratch: the Octarellin model**

C. E. Martina¹, M. Figueroa², C. Gutiérrez-Maroto¹, R. Moretti³, S. Combs³, J. Meiler³, C. van de Weerd¹, A. Matagne¹

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De novo protein design is a growing field in protein chemistry, where artificial proteins are first designed *in silico* and then validated experimentally. Our group has a long tradition in the design of artificial (β/α)₈-barrel proteins, known as Octarellins [1]. This fold, also known as TIM-barrel, is widespread in nature, particularly in enzymes, and represent an interesting target for therapeutic or biological applications. Here we present a novel protocol for the *de novo* design of TIM-barrels.

Both Rosetta and Modeller modeling softwares were used to create the backbone structure of a TIM-barrel fold and to generate more than 10.000 artificial amino acid sequences. Stability was tested for the more interesting models by running molecular dynamics (MD) simulations, using GRO-MACS. The best models were chosen for protein production and preliminary biophysical characterization.

The design of artificial proteins and the improvement of bioinformatics tools for protein modeling, structure prediction and MD simulations seem to be essential for a comprehensive knowledge of protein structure in general, as well as for an optimal use of the massive amount of data resulting from the numerous genome sequencing projects.

1. Figueroa, M. *et al.*, (2016), *J. Struct. Biol.*, 195, 19–30.

Oral Presentations

– 19. *Experimental and computational approaches to protein design* –

O-114 (P-619)**Function conversion between CPD and (6-4) photolyases**

D. Yamada^{1,2}, H. M. Dokainish³, T. Iwata¹, J. Yamamoto⁴, T. Ishikawa⁵, T. Todo⁵, S. Iwai⁴, E. D. Getzoff⁶, K. Yura², A. Kitao³, H. Kandori¹

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Photolyases (PHRs) are DNA repair enzymes with two different types: CPD PHR repairs cyclobutane pyrimidine dimers (CPDs), while (6-4) PHR repairs (6-4) photoproducts ((6-4) PPs). The features distinguishing the substrates of CPD and (6-4) PHRs are not well understood. In this study, we attempted functional conversion between CPD and (6-4) PHRs by monitoring distinct repair signals by FTIR spectroscopy. We found that a triple mutant of (6-4) PHR can repair the CPD. In contrast, the (6-4) PP was not repaired by the reverse triple mutation of CPD PHR, even after eight more mutations were added. The observed asymmetric functional conversion is interpreted in terms of more complex repair mechanism for (6-4) PP, which was supported by quantum chemical/molecular mechanical calculation. Furthermore, we scrutinized their amino acid sequences and narrowed down the amino acid positions important for distinguishing the substrates for PHRs. Specifically, we built an alignment and compared the highly conserved positions in each PHR. The differences in the conservation pattern of amino acid residues can be an explanatory cue for the difference in the function of PHRs.

Oral Presentations**– 20. Active matter –****O-115****Surface-bound enzymatic reactions organize microcapsules and protocells in solution**O. E. Shklyae¹, H. Shum¹, A. Sen², A. C. Balazs¹¹University of Pittsburgh, USA; ²The Pennsylvania State University, USA

By developing new computational models, we examine how enzymatic reactions on an underlying surface can be harnessed to direct the motion and organization of reagent-laden microcapsules in a fluid-filled microchannel. In the presence of the appropriate reagents, surface-bound enzymes can act as pumps, which drive large-scale fluid flows. When the reagents diffuse through the capsules' porous shells, they can react with enzymatic sites on the bottom surface. The ensuing reaction generates fluid density variations, which result in fluid flows. These flows carry the suspended microcapsules and drive them to aggregate into "colonies" on and near the enzyme-covered sites. This aggregation continues until the reagent has been depleted and the convection stops. We show that the shape of the assembled colonies can be tailored by patterning the distribution of enzymes on the surface.

O-117**Active nematics at interfaces**P. Guillamat, J. Ignés-Mullol, F. Sagués

University of Barcelona, Spain

Active liquid crystals are a new class of soft materials that have recently raised a huge interest. In particular, reconstituted suspensions of cytoskeletal filaments and associated motor proteins have proven ideal for studies of the origin of subcellular organization. Here we refer to the system initially engineered by the group of Z. Dogic, consisting of bundled microtubules powered by ATP-fueled kinesin motors [1]. We concentrate on two-dimensional preparations showing nematic textures and streaming flows, from largely-organized to seemingly chaotic. We will present results on different scenarios where this active nematics system is conditioned with interfacial fluids. The simplest situation corresponds to prepare them in contact with isotropic oils of different viscosities. More striking is the situation when the contacting passive fluid is a liquid crystal in its smectic phase. In this latter situation, a totally unprecedented strategy of control of the active flows has been recently demonstrated [2]. Other scenarios corresponding to encapsulated active nematics oils will be briefly presented.

[1] T. Sanchez et al., Nature 491 (7424): 431-434 (2012)

[2] P. Guillamat et al., Proc. Natl. Acad. Sci. USA, 113 (20):5498-5502 (2016)

O-116**Extensile actomyosin?**

M. Lenz

LPTMS, CNRS, Univ. Paris-Sud, Université Paris-Saclay, France

Many living organisms move thanks to nanoscopic motors that bind to and displace thin intracellular filaments. Empirically, this motion results in large-scale cellular contraction. More microscopically however, it is unclear why random pushing and pulling by the motors elicits contraction, not extension. Two main explanations for this asymmetry have been proposed, respectively based on motor self-organization and filament mechanical response, but experimental protocols to decisively discriminate between them are missing. Here propose one such test by demonstrating that self-organization robustly generates extension under certain parameter regimes, while the mechanical model can only elicit contraction. These conditions are experimentally accessible, and thus offer a promising avenue to elucidate a cornerstone of cell motility.

O-118 (P-632)**Flagella-mediated unspecific adhesion of *Chlamydomonas* to surfaces is switchable by light**C. T. Kreis, M. Le Blay, C. Linne, M. M. Makowski, O. Bäumchen

Max Planck Institute for Dynamics and Self-Organization, Germany

The natural habitats of many microorganisms such as bacteria and microalgae are liquid-infused confined geometries, e.g. the interstitial space of rocks and soil, where interactions with interfaces are of paramount importance. We performed *in vivo* micropipette force spectroscopy experiments on the unicellular biflagellated microalga *Chlamydomonas*, a prime model organism in cell- and microbiology. We discovered that the flagella-mediated unspecific adhesion to surfaces can be switched on and off by light. Single-cell micropipette experiments show that the light-switchable adhesiveness of the flagella is a completely reversible process that appears to be based on a redistribution of adhesion-promoting flagella membrane proteins on a timescale of seconds. Light-switchable adhesion enables the cell to regulate the transition between planktonic and surface-associated state, which possibly represents an adhesive adaptation to optimize the photosynthetic efficiency in conjunction with phototaxis. The kinetics of the light-induced active approach of the cell towards the surface via flagella-surface contacts is monitored in time-resolved experiments and linked to a model, taking into account the forces generated by the molecular motors under external load.

Oral Presentations**– 20. Active matter –****O-119 (P-633)****Swimming and rafting of *E. coli* microcolonies at air-liquid interfaces**G. Sinibaldi¹, V. Iebba¹, M. Chinappi¹

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The dynamics of active colloidal suspension and swimming microorganisms is strongly affected by solid-liquid and air-liquid interfaces. In this contribution, we discuss the motion of *E. coli* at an air-liquid boundary. We experimentally observed and characterized the motion of both single *E. coli* and microcolonies. Both of them follow circular trajectories. Single bacteria preferentially show a counter-clockwise motion, in agreement with hydrodynamics simulation results obtained via a boundary element method (Pimponi et al. J. Fluid Mech. 2016, vol. 789, pp. 514–533). Instead, no preferential rotation direction is observed for microcolonies suggesting that their motion is due to a different physical mechanism. We propose a simple mechanical model where the microcolonies move like rafts constrained to the air-liquid interface to partially explain the experimental data. Finally, we observed that the microcolony growth is due to the aggregation of colliding single-swimmers, suggesting that the microcolony formation resembles a condensation process where the first nucleus originates by the collision between two single swimmers.

O-120 (P-634)**Spatial confinement of active microtubule networks induces large-scale rotational cytoplasmic flow**K. Suzuki^{1,2}, M. Miyazaki^{1,2}, J. Takagi¹, T. Itabashi^{1,2}, S. Ishiwata¹

¹Department of Physics, Faculty of Science and Engineering, Waseda University, Japan; ²Waseda Bioscience Research Institute in Singapore (WABIOS), Singapore

Collective behaviors of motile units through hydrodynamic interactions induce directed fluid flow on a larger length scale than individual units. The motor-driven elongation of microtubule (MT) bundles generates turbulent-like flow in purified systems; however, it remains unclear whether and how MT bundles induce large-scale directed flow like the cytoplasmic streaming observed in cells. Here, we adopted *Xenopus* egg extracts as a model system of cytoplasm and found that MT bundle elongation induces directed flow for which the length scale and timescale depend on the existence of geometrical constraints. At the lower activity of dynein, kinesins bundle and slide MTs, organizing a random network of extensile MT bundles. When the extracts were encapsulated in droplets, the extensile bundles pushed the droplet boundary. This force initiated symmetry breaking of the randomly oriented bundle network, leading to bundles aligning into a rotating vortex structure. This vortex induced rotational cytoplasmic flows on the length scale and timescale that were 10- to 100-fold longer than the vortex flows emerging in bulk extracts. Our results suggest that MT systems use not only hydrodynamic interactions but also mechanical interactions to induce large-scale temporally stable cytoplasmic flow.

Oral Presentations**– 21. Membrane permeation: transporters –****O-121****Structural basis for proton coupled amino acid and peptide transport**

S. Newstead

University of Oxford, UK

Peptide transport is one of the main routes through which cells obtain nitrogen and amino acids for metabolism and growth. The POT family of membrane transporters use the inwardly directed proton electrochemical gradient to drive the uptake of peptides into the cell. Originally discovered in bacteria, members of the family have been found in all kingdoms of life except the archaea. A remarkable feature of the family is their diverse substrate promiscuity. Whereas in mammals and bacteria they are predominantly di- and tri-peptide transporters, in plants the family has diverged to recognise nitrate, plant defence compounds and hormones. This promiscuity has led to the development of peptide-based pro-drugs that use PepT1 and PepT2, the mammalian homologues, to improve oral drug delivery. Recent crystal structures from bacterial and plant members of the family have revealed conserved features of the ligand-binding site and provided insights into post-translational regulation. In this talk I will discuss our current understanding of transport, ligand promiscuity and regulation within the POT family.

O-123**Structural and mechanistic investigation of the human glucose transporters GLUTs**

N. Yan

Center for Structural Biology, Tsinghua University, Beijing, China

Glucose is the primary fuel to life on earth. Cellular uptake of glucose is a fundamental process for metabolism, growth, and homeostasis. The major facilitator superfamily glucose transporters GLUTs, exemplified by human GLUT1-4, are prototypes in the study of solute transport. We were able to determine the atomic structures of human GLUT1 and GLUT3 in multiple conformations during a transport cycle, which reveal the molecular basis for ligand recognition and transport. The 1.5 angstrom structure of GLUT3 in complex with glucose reveals the molecular details of substrate coordination. The crystal structure of human GLUT1 at 3.2 angstrom resolution in the inward-open conformation allows accurate mapping and potential mechanistic understanding of disease-associated mutations in GLUT1. Comparison of the GLUT structures in the outward-open, outward-occluded, and inward-open states provides insights into the alternating access cycle for GLUTs, whereby the C domain provides the primary substrate binding site and the amino terminal domain undergoes rigid-body rotation with respect to C domain. We also determined the crystal structure of XylE, an *E. coli* homologue of GLUT1-4. Whereas GLUT1-4 are facilitative uniporters, XylE is a proton-driven symporter. Structural comparison and biochemical analysis of GLUTs and XylE allow examination of transport mechanisms by passive facilitators versus active transporters.

O-122**Protein-lipid interplay revealed by X-ray solvent contrast modulation with calcium pump crystals**

C. Toyoshima, Y. Norimatsu

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan

Our knowledge of membrane proteins is rapidly increasing and the idea that transmembrane helices of a membrane transport protein may rearrange substantially during the reaction cycle is now well established. However, our understanding of phospholipid- and protein-phospholipid interactions are severely limited, because the lipid bilayer has eluded visualisation by conventional crystallographic methods. We now know, through crystallographic studies on Ca^{2+} -ATPase in various states, that the lipid bilayer has an important mechanistic role in achieving pump functions. We have developed X-ray solvent contrast modulation for visualization of the lipid bilayer and applied it to the crystals of Ca^{2+} -ATPase in four different states. The electron density maps thus obtained resolve entire first layer phospholipids surrounding the transmembrane helices, although less than half of them are hydrogen bonded to protein residues. These maps show different roles of Arg/Lys and Trp residues in interacting with the bilayer for achieving pump functions, and, presumably, in dynamics of membrane proteins in general.

O-124 (P-649)**Using bacteria to fight bacteria: Parasitisation of ferredoxin-uptake receptors in *Pectobacterium***C. Thompson¹, R. Grinter², D. Walker¹, O. Byron¹¹University of Glasgow, UK; ²Monash University, Australia

Antibiotic resistance is a global problem affecting both health and food security. Gram-negative plant pathogens, such as *Pectobacterium*, have detrimental effects on crop yields. Bacteriocins are protein antibiotics that are usually active only against bacteria closely related to the producing strain; these bacteriocins are often species specific and therefore offer an alternative to the current antibiotics used in clinical practice. Three recently described bacteriocins produced by *P. carotovorum* consist of a [2Fe-2S] plant-type ferredoxin domain fused to a bacteriocin cytotoxic domain. These parasitize an existing ferredoxin uptake system to gain entry into target cells. The normal physiological role of this uptake system is to acquire iron from ferredoxin. We have identified the ferredoxin/pectocin receptor as a TonB-dependent receptor that we have designated FusA. Bioinformatic analysis indicates the existence of a ferredoxin uptake operon, which in addition to the receptor FusA, encodes a protease, an ABC-transporter which likely act to cleave ferredoxin and transport liberated iron to the cytoplasm and a homologue of TonB. We are currently dissecting the roles of these proteins in ferredoxin uptake using a combination of structural (SANS and SAXS) and functional studies.

Oral Presentations**– 21. Membrane permeation: transporters –****O-125 (P-650)****Membrane protein diffusion in living *E. coli*: from fundamentals to insight in protein translocation**

Y. J. Bollen, A. Varadarajan, F. Oswald, H. Lill, E. J. Peterman

Vrije Universiteit Amsterdam, Netherlands

The organization of the bacterial membrane has been challenging to analyze due to the small size and non-flat geometry of bacterial cells. We have used single-molecule fluorescence microscopy and three-dimensional quantitative analyses in live *Escherichia coli* to demonstrate that its cytoplasmic membrane contains microdomains with distinct physical properties. We showed that the stability of these domains depends on the integrity of the MreB cytoskeletal network. Cytoskeleton and membrane affect trans-membrane protein (TMP) diffusion. The mobility of the TMPs tested is subdiffusive, most likely caused by confinement of TMP mobility by the submembranous MreB network.

We then aimed to understand how the twin-arginine translocation (Tat) system transports fully folded proteins across the cytoplasmic membrane of bacteria. The pore-forming subunit TatA reversibly associates with substrate-binding TatBC complexes. We tracked individual eGFP-fused TatA complexes and showed that large, stable TatA complexes switch between fast and slow diffusion, with diffusion coefficients ~10-fold different. Mechanistic consequences are discussed.

O-126 (P-651)**An emerging technique for the characterization of transport proteins: SSM-based electrophysiology**

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In the past 10 years, Solid Supported Membrane (SSM)-based electrophysiology has been proven as an efficient tool for the characterization of electrogenic membrane proteins, e.g. transporters, ion pumps and ion channels. It's a label-free, high-sensitivity method which allows the use of membrane preparations, e.g. reconstituted protein samples and membranes from native tissues or cell culture. The high sensor stability allows for different solution exchange experiments using the same sensor. Both transport and binding can be resolved and kinetic parameters like rate constants, KD, KM or IC50 can be determined in a fast and easy workflow. Until now more than 100 different proteins have been tested; almost 100 peer reviewed papers have been published. Here we show data for several targets revealing different aspects of their transport mechanism. The organic cation transporter OCT2 transports multiple substrates with different EC50 and Vmax. The proton-coupled peptide transporter PEPT1 was used to show an inhibition assay. We analyzed different transport modes of the Na⁺/Ca²⁺ exchanger NCX and assayed binding and transport reactions of different sugar transporters individually. Moreover results for the Na⁺/K⁺-ATPase and the nicotinic acetylcholine receptor (nAChR) are shown.

Oral Presentations**– 22. Imaging the cell –****O-127****Advances in adaptive optics for microscopy and nanoscopy**M. J. Booth^{1,2,3}¹Department of Engineering Science, University of Oxford, UK; ²Centre for Neural Circuits and Behaviour, University of Oxford, UK; ³School in Advanced Optical Technologies, Friedrich-Alexander Universität Erlangen-Nürnberg, Germany

Adaptive optics (AO) has been introduced into microscopes in order to overcome the problems caused by specimen-induced wavefront aberrations. This is particularly important when focusing deep into tissue where the cumulative effect of focusing through the refractive index structure of the specimen causes significant wavefront distortion. These AO systems employ a dynamic element, such as a deformable mirror or spatial light modulator, to correct aberrations, restoring image quality. Adaptive optics has been demonstrated in a range of microscope modalities including conventional widefield microscopes as well as laser scanning systems with various applications in biomedical imaging and other areas. Adaptive microscopy has most recently been developed for super-resolution microscopes – or nanoscopes – which enable resolutions smaller than the diffraction limit of light. We report on a range of recent advances in this field.

O-129**Cryo X-ray tomography: 3D cellular ultrastructure of intact cells without fixation or staining**

B. Kepsutlu, P. Guttman, S. Werner, G. Schneider, J. McNally

Helmholtz-Zentrum Berlin, , Institute for Soft Matter and Functional Materials, Berlin, Germany

Soft X-ray tomography enables 3D nanoscale imaging of intact, unstained biological cells in their near native state, subject only to cryo-preservation. When cells are imaged with X-rays of 2 nm wavelength, organic matter becomes strongly absorbent and so sub-cellular organelles become readily visible without chemical staining. Here we use this X-ray imaging approach to compare cellular uptake and processing of two different nanomedicines, namely 50 nm gold nanoparticles coated with two different bioactive agents. We find similarities and differences depending on the nanoparticle coating, demonstrating that there is not a generic nanoparticle processing pathway in cells. Furthermore, using a 3D quantitative analysis of sub-cellular organellar distributions, we find that the nanomedicines have substantial effects on sub-cellular ultrastructure, which again are dependent on the nanoparticle coating. Specifically, one coating (polyethyleneimine) induces a temporary, but nearly complete loss of lysosomes while another coating (polyglycerol-sulfate) induces a temporary, but nearly complete loss of multivesicular bodies. In addition, by quantifying the delivery rates of nanoparticles to the cytoplasm and nucleus, we find that these are also dependent on the coating. In sum, our results demonstrate the power of cryo X-ray tomography for quantitative 3D analysis of cell ultrastructure, and in particular for analysis of nanomedicine delivery.

O-128**Using cryo-electron tomography to determine protein structures within complex environments**J. A. G. Briggs^{1,2}¹MRC Laboratory of Molecular Biology, Cambridge, UK;²European Molecular Biology Laboratory, Heidelberg, Germany

Electron microscopy is undergoing a revolution. New and improved hardware and software are allowing researchers to generate images of unprecedented quality. It is possible to use cryo-electron microscopy to determine the structures of purified protein complexes at atomic resolution, as well as to get detailed views of biological objects within cells and tissues.

We are studying the structure and assembly of enveloped viruses like HIV, as well as the machinery involved in membrane trafficking within the cell. By combining cryo-electron tomography with computational image processing, we can visualize the structures of proteins assembled on heterogeneous membrane systems in vitro with sufficient resolution to determine protein structure *ab initio*. The same methods can be used to determine the structures of assembled protein complexes directly within cells.

I will discuss the potential of the technology, and present our most recent data on the structures of viruses and coated vesicles in vitro and within cells.

O-130 (P-667)**Application of indirect optical micromanipulation in fluorescent 3D live cell imaging**

G. Vizsnyiczai, A. Buzás, B. L. Aekbote, T. Fekete, I. Grexa, P. Ormos, L. Kelemen

Biological Research Centre, Hungary

Optical micromanipulation of live cells has been extensively used to study cellular phenomena in basic research or in diagnostics frequently coupled with advanced imaging techniques. Photodamage, often associated with direct cell trapping can be reduced with the careful choice of the laser wavelength. Alternatively, using cell-attached intermediate objects as handles could practically eliminate the irradiation damage and increase trapping efficiency due to the high refractive index contrast. Here we introduce the technique of indirect optical micromanipulation using shape-optimized intermediate objects made by two-photon polymerization and enabled by holographic optical tweezers. With this technique the cell-to-focal spot distance is increased to several micrometers while maintaining the cells rapid maneuverability with 6 degrees of freedom. We demonstrate the power of the method with 3D fluorescent live cell imaging. We achieved 3D reconstruction of single cells with isotropic resolution by imaging them at different orientations. The various views of the cells were achieved by rotating them around an axis perpendicular to the optical axis with the optical tweezers. The presented tool and manipulation scheme can be readily applied in a range of optical microscopic techniques.

Oral Presentations**– 22. Imaging the cell –****O-131 (P-668)****Transcription factor clusters regulate gene expression in yeast *Saccharomyces cerevisiae***S. Shashkova^{1,2}, A. J. M. Wollman¹, E. G. Hedlund¹, S. Hohmann^{2,3}, M. C. Leake¹¹Biological Physical Sciences Institute, University of York, York YO10 5DD, UK; ²Department of Chemistry and Molecular Biology, University of Gothenburg, 40530 Göteborg, Sweden; ³Department of Biology and Biological Engineering, Chalmers University of Technology, 41296 Göteborg, Sweden

In yeast *S. cerevisiae* expression of genes essential for alternative carbon sources metabolism is regulated by the transcription factor (TF) Mig1. Upon glucose limitation, Mig1 becomes phosphorylated and relocates to the cytoplasm. Glucose causes Mig1 dephosphorylation and import to the nucleus where it binds to target promoters mediating gene repression. However, the exact mechanism of gene regulation by Mig1 is unclear. We combined live-cell single-molecule imaging with traditional biochemical methods to study Mig1 localisation and phosphorylation dynamics. Our data show that Mig1 is present as monomer and oligomers in the cytoplasm and nucleus, constantly shuttling between these compartments regardless of glucose presence. We found that Mig1 phosphorylation is not solely regulated by glucose and does not drive Mig1 translocation across the nuclear membrane. Mapping Mig1 target promoter sequences onto a 3D yeast chromosomes structure model allowed us to model the 3D arrangement of Mig1 in the nucleus and was consistent with Mig1 clusters. Studies on another TF showed similar oligomeric organisation. We suggest that yeast gene regulation is mediated via TFs which act as multimeric clusters.

O-132 (P-669)**Pair correlation analysis of fixed PALM and live PALM applied on the water channel AQP3**E. C. Arnsperg^{3,2}, P. Sengupta^{2,4}, H. H. Jensen¹, U. Hahn¹, I. T. Andersen¹, E. B. Jensen¹, K. Mortensen⁵, J. Lippincott-Schwartz^{2,4}, L. N. Nejsun¹¹AU, Denmark; ²NIH, USA; ³SDU, Denmark; ⁴Janelia Research Campus, USA; ⁵DTU, Denmark

Water transport across the plasma membrane is mediated by aquaporin (AQP) water channels. We previously found that a short-term increase in cAMP leads to an increase in lateral diffusion of AQP3, revealing short-term regulation. To further study if AQP3 is regulated at the nanoscale, we applied pair correlation in combination with PALM. This showed that AQP3 organize in nano-domains smaller than 60 nm and upon cAMP stimulation, changed organization to 60 – 200 nm sized nano-domains. Thus, PC-PALM revealed regulation at the nanometer resolution. Furthermore, we performed live-PALM of AQP3 upon cAMP stimulation. Power-spectral analysis of the single-molecule trajectories revealed that the molecules were not freely diffusing. Rather, data were consistent with a simple model for 2D diffusion in confinement. While the measured diffusion coefficients of AQP3 were identical between control and cAMP stimulated cells, the confinement radius increased significantly. Thus fixed and live PALM measurements both revealed a change of AQP3 nano-organization in the plasma membrane upon cAMP stimulation, indicating short-term hormone regulation of AQP3 at the nanoscale level which has so far been undetectable.

Oral Presentations**– 23. Biomimetic structures and systems –****O-133****Precision measurements of biomolecular interactions and structure, supported by DNA origami**

H. Dietz

Technische Universität München, Germany

Programmable self-assembly with DNA origami allows creating custom-shaped nanoscale objects. Through this capacity, DNA origami enables constructing custom instruments to perform precision measurements of molecular interactions and structure, with enhanced control over positioning, orientating and manipulating the molecules under study. In my presentation I will report about a series of experiments in which we exploited this capacity to dissect the weak stacking forces between individual basepairs (1) and the forces that act between pairs of nucleosomes (2), and discuss some more recent results.

(1) F. Kilchherr, C. Wachauf, B. Pelz, M. Rief, M. Zacharias, and H. Dietz, 'Single-molecule dissection of stacking forces in DNA' *Science*, (2016), doi 10.1126/science.aaf5508

(2) J. Funke, P. Ketterer, C. Lieleg*, S. Schunter, P. Korber, and H. Dietz, 'Uncovering the forces between nucleosomes using DNA origami', *Science Advances*, (2016), doi 10.1126/sciadv.1600974

O-135**Building life-like systems: artificial cells and organelles**R. Peters², L. K. E. A. Abdelmohsen¹, B. Buddingh¹, D. Williams¹, F. Peng², M. Nijemeisland², J. C. van Hest^{1,2}

¹Eindhoven University of Technology, Department of Chemical Engineering and Chemistry, Netherlands; ²Radboud University, Institute for Molecules and Materials, Netherlands

In nature many biological processes are compartmentalized to ensure their integrity and efficiency. Inspired by this phenomenon, we develop capsules by combining enzymes and amphiphilic block copolymers into bioactive compartments. Intrinsically porous enzyme-loaded polymer vesicles were explored as artificial organelles. These nanoreactors were introduced in living cells and showed to be catalytically active. We furthermore investigated the encapsulation of multiple nanoreactors in a larger polymersome, to mimic the structural build-up of a eukaryotic cell.

One important aspect of living systems is that they can communicate with and respond to changes in the environment. We have been able to build a synthetic cell in which an internal biocatalytic process was triggered by the external addition of small molecules.

Living cells are able to move via chemotaxis. We have applied this concept to bowl-shaped indented vesicles, stomatocytes. Enzymes were encapsulated that produced oxygen. Due to their anisotropic shape, the catalytic activity allowed the particles to move. By employing a chemical gradient chemotaxis was observed. We have also constructed an enzyme network, which allows us to regulate the speed of the nanomotors.

O-134**Synthetic genetics: Beyond DNA and RNA**

P. Holliger

MRC Laboratory of Molecular Biology, Cambridge, UK

Synthetic biology seeks to probe fundamental aspects of biological form and function by construction (i.e. resynthesis) rather than deconstruction (analysis). Synthesis thus complements analytical studies of life, and allows novel approaches towards fundamental biological questions.

We have been exploiting the synthesis paradigm to explore the chemical etiology of the genetic apparatus shared by all life on earth. Specifically, we ask why information storage and propagation in biological systems is based on just two types of nucleic acids, DNA and RNA. Is the chemistry of life's genetic system based on a "frozen accident", imposed at the origin of life, or are DNA and RNA functionally superior to simple alternatives?

I'll be presenting progress in the engineering of polymerases for the replication and evolution of novel synthetic genetic polymers, which we term XNAs. We show that eight different synthetic polymers, based on nucleic acid architectures not found in nature, can mediate genetic information storage and propagation. We demonstrate a capacity for Darwinian evolution by the *de novo* selection of specific ligands (XNA aptamers) and catalysts (XNAzymes) from random XNA sequence repertoires. Finally, encoded XNA synthesis allows the assembly of simple XNA nanostructures.

O-136 (P-700)**Characterization of matrix vesicles biomimetic systems: interaction with collagen fibers during biomineralization**P. Ciancaglini¹, M. Bolean¹, A. M. S. Simão¹, M. Bottini^{2,3}, J. L. Millán³

¹USP, Ribeirão Preto, Brazil; ²University of Rome Tor Vergata, Italy; ³SBP Medical Discovery Institute, USA

Osteoblasts control the deposition of the extracellular matrix and the release of matrix vesicles (MVs), which serve as the initial sites for hydroxyapatite formation. Annexin V (AnxA5) is a phospholipid-dependent Ca^{2+} -binding protein, which acts as Ca^{2+} -channel in the MVs' membrane. Tissue-nonspecific alkaline phosphatase (TNAP) is attached to the MVs' outer membrane and acts as a pyrophosphatase and ATPase producing P_i and regulating the mineralization. We describe the preparation of DPPC and DPPC:DPPS (9:1) proteoliposomes harboring AnxA5, TNAP or AnxA5+TNAP, and their use as MVs mimetic systems. Enzymatic activity and Ca^{2+} uptake validated the functional incorporation of both proteins in mimetic systems. AnxA5-proteoliposomes bound type II collagen with the highest affinity when compared with collagen I and III. The presence of DPPS significantly enhanced the binding up to 74%. TNAP-proteoliposomes for both lipid compositions poorly bound the collagen matrix (< 20%). Proteoliposomes harboring both proteins showed 30% of binding. The interaction between AnxA5 and type II was Ca^{2+} independent. These findings suggest that AnxA5 has a double role, create a Ca^{2+} -rich environment inside MVs and anchor MVs to collagen at calcification sites. CNPq, CAPES, FAPESP.

Oral Presentations**– 23. Biomimetic structures and systems –****O-137 (P-698)****Protein assembly, from small molecule to polymer mediators**

P. B. Crowley

NUI Galway, Ireland

Protein assembly triggered by supramolecular building blocks offers fascinating routes to hybrid bio-inspired materials. We are using calixarenes as “molecular glues” to mediate protein assembly. A combined methodology of NMR spectroscopy, X-ray crystallography and SEC-MALS has provided convincing evidence that sulfonato-calix[n]arenes are versatile ligands for protein surface recognition and assembly.[1–3] New examples of calixarene-mediated assembly will be presented.

Recently, we showed that PEGylation can result in highly porous protein assemblies.[4] Current efforts are focused on protein interactions with PEGylated-calixarenes, which also yield porous assemblies as revealed by X-ray crystallography.

[1] McGovern, Fernandes, Khan, Power, Crowley, *Nature Chem.* **2012**, 4, 527.

[2] McGovern, McCarthy, Crowley, *Chem. Commun.* **2014**, 50, 10412.

[3] Rennie, Doolan, Raston, Crowley, *Angew. Chem.* **2017**, *In press*.

[4] Cattani, Vogeley, Crowley, *Nature Chem.* **2015**, 7, 823.

O-138 (P-699)**Super-resolution DNA-origami barcodes: a labeling system for spatially resolved deep-sequencing**

F. Ferenc, I. Masakazu, B. Erik, H. Björn

Karolinska Institutet, Sweden

It has been demonstrated with the emergence of single-cell sequencing techniques that adjacent cells in tissues can show a remarkable diversity in terms of gene activity, which was previously impossible to detect with bulk experiments. One of the current main efforts of the single cell transcriptomics field is to link spatial information to single cell transcription data. In the presented work, we describe the use of DNA-origami for the development of a multiplexable tagging-system, detectable by next-generation sequencing (NGS) as well as super-resolution microscopy, with the potential to uniquely label a great number of cells in tissue-samples and permitting the coupling of the RNA-seq data of individual cells to positional information through the use of microscopy images of barcoded samples. The described tagging-system consist of a library of geometrically encoded, super-resolution DNA-origami barcodes, constructed in a combinatorial fashion, that can be read out using super-resolution microscopy and NGS techniques as well, through the encoding of the barcodes' optical code into the sequence of their scaffold DNA molecules. The labeling-system is aimed to be adapted as a preceding labeling and imaging step to the already established single-cell RNA-seq protocols.

Oral Presentations**– 24. Drug discovery and delivery –****O-139****Phase contrast single particle analysis of small protein complexes at atomic resolutions**M. Khoshouei¹, L. Liang², M. Radjainia³, W. Baumeister¹, R. Danev¹¹Max-Planck Institute of Biochemistry, Germany; ²Monash Institute of Pharmaceutical Sciences, Australia; ³Thermo Fisher Scientific (formerly FEI), Netherlands

Here we demonstrate the ability of the Volta phase plate which enables contrast enhancement and structure determination of small protein complexes at atomic resolutions. At the current status of conventional Cryo-EM some challenging complexes such as small proteins (smaller than 100 kDa) and/or the ones which are not crystallised are still on the way to be analysed. Phase plate technology is a technique which holds a promise in bringing up atomic resolution structures of the mentioned complexes. Moreover, important and challenging drug targets could be potential specimens to be investigated by the Volta phase plate in order to take an advantage in structure-based drug design.

O-141**Mechanisms of microbubble mediated drug delivery**M. Aron¹, V. Pereno¹, D. Carugo¹, O. Vince¹, M. K. Kuimova², J. Bernadino de la Serna¹, E. Sezgin¹, C. Eggeling¹, E. P. Stride¹¹University of Oxford, UK; ²Imperial College, UK

There is now a substantial literature demonstrating the potential of surfactant stabilised microbubbles as drug delivery agents; particularly in combination with therapeutic ultrasound. Microbubbles provide a means of encapsulating drugs to avoid interaction with healthy tissue. They can be functionalized to enable them to be targeted to a specific tissue volume and drug release can be controlled temporally and spatially through exposure to a focused ultrasound beam. In addition, the biophysical and biochemical effects produced by an oscillating bubble can significantly enhance both the distribution of a drug within the tissue and its uptake at a cellular level. The underlying mechanisms however are still poorly understood. This talk will provide an overview of the relevant phenomena and their role in microbubble mediated delivery. The results of the authors' recent investigations into the role of microbubble composition and of inter and intracellular streaming will also be presented.

O-140**Fragment screening using native state mass spectrometry**

S.-A. Poulsen

Griffith Institute for Drug Discovery (GRIDD), Griffith University, Australia

Fragment based drug discovery (FBDD) is a recently validated approach to identify small molecules as better chemical starting points for drug discovery. Since 2005, fragment screening has resulted in two FDA approved drugs and more than 30 drug candidates in clinical trials. The take-up of FBDD in academia, biotech and pharma is growing owing to this success.

FBDD is contingent on the development of robust analytical methods to identify weak, noncovalent protein–fragment interactions. In contrast to high throughput screening (HTS), where hit compounds are relatively potent (K_D s in the nM to μ M range), the binding interactions of hit fragments tend to be considerably weaker (K_D s in the μ M to mM range), so that fragment hits may not be as readily identified in classical biochemical screens as for HTS hits.

A number of biophysical techniques have been used to screen fragment libraries with some of the most popular techniques being NMR, SPR and X-ray crystallography. The use of mass spectrometry for fragment screening has remained relatively underexplored. This presentation will highlight the attributes of mass spectrometry as a complementary screening method in fragment-based drug discovery. Specifically we will discuss the discovery of a new zinc binding chemotype.

O-142 (P-749)**Intrinsic vs. observed thermodynamic and kinetic parameters of carbonic anhydrase-ligand interaction**V. Linkuviene¹, V. O. Talibov², H. Danielson^{2,3}, D. Matulis¹¹Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Lithuania;²Department of Chemistry - BMC, Uppsala University, Sweden;³Science for Life Laboratory, Uppsala University, Sweden

Drug design is often based on a structure-activity relationship analysis. Different groups of a compound can show various binding affinity and association-dissociation rates to a target protein. However, the observed protein-ligand binding parameters do not reveal the influence of other contributing reactions in this interplay.

We use carbonic anhydrase (CA), vital enzymes that catalyze carbon dioxide hydration. But misregulation of their expression can cause diseases, such as epilepsy, cancer, etc. Therefore CA inhibitors are of interest in pharmaceutical research, but since CAs have highly similar structure of the active site, it is difficult to design compounds that would selectively inhibit one isoform.

CA – compound interaction was determined by the fluorescent thermal shift assay, isothermal titration calorimetry, and surface plasmon resonance. Combination of parameters, which can be obtained by three methods, provided the direction of optimization of the compound binding affinity and selectivity towards the desired CA isoform.

Oral Presentations**– 24. Drug discovery and delivery –****O-143 (P-750)****Development and characterization of polymeric nanoparticle**

B. F. C. Patricio¹, J. M. Pietra², C. Martinez², S. D. V. Alonso², T. Barboza³, S. A. Lopes³, P. M. Bisch¹, G. Weissmüller¹

¹Laboratório de Física Biológica, IBCCF, CCS, UFRJ, Brazil; ²Laboratório de Biomembranas, UNQ, Argentina; ³Departamento de radiologia, HU-UFRJ, Brazil

Nanotechnology aim to solve several pharmacological problems. To guarantee the success of nanoparticles (NP) it is important to ensure homogenous particle size, surface hydrophilicity and prolonged circulation time. This work aimed to produce and evaluate PLA/PVA and PEG/PLA/PVA NP with different size and surface properties and evaluated it by *in vivo* test. The NP were produced by double emulsion method with changes in both power and sonication time to obtain NP with different sizes. Using the Zebrafish WT model (CE-UNQ 2/2014) it was possible to observe that the PLA/PVA NP were more cardiotoxic than those pegylated. Furthermore, hepatotoxicity and neurotoxicity were higher in large pegylated NP. Finally, both NP were labeled with ^{99m}Tc and injected into Wistar rats (CEUA 086/15). It was obtained gamma camera images and the organs activity were measured. It was possible to define that pegylation decreased the uptake of the larger NP by the liver, but do not change the uptake for the smaller ones. The non-pegylated NP accumulated in the lungs. In this work, it was possible to determine the better conditions for production of NP. Also, it allows the understanding of the toxicity and biodistribution of the nanoparticles at *in vivo* models.

O-144 (P-751)**Anti-transferrin receptor antibody conjugated PLGA nanoparticles for temozolomide delivery**

M. J. Ramalho¹, J. A. Loureiro¹, J. Lima², M. A. N. Coelho¹, M. C. Pereira¹

¹LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Portugal; ²Cancer Signaling and Metabolism Group, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

The classical temozolomide (TMZ) treatment for glioblastoma is rarely curative, due to TMZ's poor ability to cross the blood-brain barrier (BBB) [1]. Thus, improved delivery systems may be a suitable strategy to overcome those limitations. TMZ was encapsulated in poly(lactide-coglycolide) nanoparticles (PLGA NPs), and dual-targeting approach was envisaged using NPs modified with anti-transferrin receptor monoclonal antibody (mAb), since this receptor is overexpressed in BBB and cancer cells [2]. The PLGA NPs showed mean diameters of 200 nm and encapsulation efficiency of 45%. Release studies showed a lower diffusion rate of TMZ from mAb-PLGA NPs when comparing with non-modified NPs. The mAb layer on the surface of PLGA NPs may hinder water permeation, resulting in a lower diffusion. The cytotoxicity of TMZ-PLGA NPs was evaluated on two human glioma cell lines, U251 and U87, demonstrating that the antineoplastic effect of TMZ is enhanced by the nanoformulation. However, cytotoxic effect of TMZ was to some extent decreased with the mAb-modification, due to the lower release rate of the drug. Further studies will confirm whether the use of the mAbs will present advantages in *in vivo* conditions, allowing increased transport across the BBB.

Oral Presentations**– 25. Motility and migration –****O-145****Actin turnover in motile cells**

K. Keren

Physics Department, Technion – Israel Institute of Technology, Haifa, Israel

Actin turnover is the central driving force underlying cell motility. The molecular components involved are largely known, and their properties have been studied extensively *in vitro*. However, a comprehensive quantitative picture of actin turnover *in vivo* is still missing. We focus on lamellipodial fragments from fish epithelial keratocytes, which lack the cell body but retain the ability to crawl with speed and persistence similar to whole cells. The geometric simplicity of fragments and the absence of additional actin structures allow us to characterize the spatio-temporal actin organization in their lamellipodium with unprecedented detail. These experimental measurements serve to guide the development of a predictive quantitative model of actin turnover in motile lamellipodia. Our results indicate that the bulk of the cytoplasmic actin pool is not available for polymerization, allowing diffusion to recycle actin effectively and facilitate steady cell migration, while maintaining the cell's ability to generate rapid focused acceleration when needed.

O-147 (P-790)**4D fast quantitative imaging of vascular invasion: the role of cell-matrix mechanical interaction**C. Steuwe^{1,2}, M.-M. Vaeyens², A. Jorge-Peñas², M. Roeffaers¹, H. van Oosterwyck^{2,3}

¹Centre for surface chemistry and catalysis, KU Leuven, Belgium; ²Biomechanics Section, KU Leuven, Belgium; ³Prometheus-Mechanobiology, KU Leuven, Belgium

Tissue engineering aims at recapitulating development, growth and regeneration of tissues and organs. It is therefore vital to assess multicellular processes in a fast, quantitative and non-invasive way. Here we present an approach to study sprouting angiogenesis in 3D with high temporal resolution and under chemically defined culture conditions. Multimodal and fast lightsheet microscopy was used to acquire stacks of markers during the live sprouting of human umbilical vein endothelial cells and after their chemically induced relaxation. The calculation of the matrix deformations was formulated as a 3D non-rigid image registration process and is based on data either from beads or from fibers.

Using this technique we deduct highly temporally resolved mappings of traction-induced matrix deformations of a 3D collagen matrix around angiogenic sprouts. It allows to gain quantitative understanding on rapid mechanisms that govern multi- and intercellular dynamics under various conditions.

O-146**Rigidity sensing is regulated by receptor tyrosine kinases to inhibit transformed growth**B. Yang¹, H. Wolfenson², S. Liu¹, Z. Z. Lieu¹, F. M. Hameed¹, A. D. Bershadsky¹, M. P. Sheetz^{1,2}

¹Mechanobiology Institute, National University of Singapore, Singapore; ²Department of Biological Sciences, Columbia University, New York, USA

Matrix rigidity is an important physical aspect of cell microenvironments; however, the mechanism by which cells test substrate rigidity is not clear. Submicron pillar studies indicate that cells sense rigidity by measuring the forces required for local standard contractions at the cell periphery (pinching activity) (Ghassemi et al., 2012. PNAS 109:5328). Recent observations show that sarcomere-like units drive step-wise contractions that depend upon tropomyosin to sense rigidity and block growth on soft surfaces (Wolfenson et al., 2016. Nat. Cell Bio. 18:33). The RTKs, AXL and ROR2, involved in cancer progression are part of the contractile units and control distance and duration of contractions (Yang et al., 2016. Nano Lett 16(9): 5951). In addition, EGFR and HER2 control rigidity sensing only on rigid surfaces without ligand (Liu et al., in press). Thus, we suggest that RTKs affect adhesion-dependent cell transformation and morphology changes in development through their regulation of local mechanosensory contractions by sarcomere-like units with tropomyosin.

O-148 (P-791)**Instabilities of competing tissues with mechanically-cued proliferation**J. J. Williamson, G. Salbreux
The Francis Crick Institute, UK

Inspired by tissue replacement processes such as in the developing abdominal epidermis of *Drosophila*, we model a propagating interface between competing epithelial tissues in which cell division and death are mechanically cued. We derive the steady propagation in 1D and show how this can be driven either by an imbalance in “homeostatic pressure”, or by active, directed motility forces present in one or both tissues. Then, we determine whether the interface is stable against stochastic fluctuations of its contour. We find that a number of destabilising effects are possible. One of these involves the substrate friction and is reminiscent of the Saffman-Taylor or “viscous fingering” instability, which occurs in non-living systems and is well-known in oilfield engineering. The interface can also become unstable on longer length scales via an effective viscosity associated with cell division and death. Active motility forces play a complex role, through which even a stationary interface may be unstable or stable depending on the direction of motility forces in each tissue. Our results provide insight into instabilities arising purely from the effective material parameters of mechanosensitive tissues.

Oral Presentations*– 25. Motility and migration –***O-149 (P-635)****Spontaneous and induced gait-switching in microswimmers**

K. Y. Wan, R. E. Goldstein
University of Cambridge, UK

Self-propulsion by structures known as cilia and flagella presents a significant selective advantage. Great variability exists in the number of flagella, their beating modes, and the basal architecture whence the flagella emanate. In certain bacteria, flagella bundle behind a rod-shaped cell to push the organism forward, while the model alga *C. reinhardtii* uses two near-identical flagella to pull itself through the fluid, executing a breaststroke. In reality, neither gait is stereotypical. For free-living unicellular eukaryotes with few flagella the question of their actuation and coordination has been receiving growing attention from theorists and experimentalists alike. Performing a comparative study across select flagellates, we demonstrate an unprecedented diversity in swimming gaits and reveal the extent to which control of flagellar motility is driven intracellularly. Stochastic bifurcations between different modes of swimming are visualised at high spatiotemporal resolution, and dynamic changes in flagellar beating shown to elicit in trajectory reorientation and responsive navigation at the level of individual cells. These insights suggest that the capacity for fast transduction of signal to peripheral appendages may have evolved far earlier than previously thought.

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Oral Presentations**– 3Rs and Biophysics –****O-150****Using the breast cancer now tissue bank to generate new biomaterials to accelerate breast cancer research and address the 3Rs**

V. Speirs

Leeds Institute of Cancer and Pathology, University of Leeds, UK

The Breast Cancer Now Tissue Bank (BCNTB) was established in 2010 to meet the needs of the breast cancer research community who recognised that access to a source of carefully collected well-annotated human breast tissue was essential for translational research.

BCNTB is a unique resource of biological materials and supportive clinical data, efficiently and ethically collected from patients with breast cancer, to provide researchers with high quality, relevant materials, helping to raise the standard of breast cancer research and facilitating the co-ordinated translation of scientific findings into the clinical setting.

A wide range of biological materials are banked, including fresh frozen tumour and surrounding tissue, whole blood and serum samples, as well as formalin-fixed paraffin-embedded material. A unique feature of the Bank is its cell culture programme which offers researchers a wide range of isolated purified cell populations, including explants, organoids, purified epithelial and myoepithelial cell fibroblasts. This provides researchers new ways to model breast cancer without the need to use animals. As science is constantly evolving the Bank has an inbuilt R&D program, including cell immortalisation, investigating improved sample storage and collection methods and on-going IT development, all of which will ensure the bank remains cutting-edge.

O-152**Using small non-antibody binding proteins to visualise the cytoskeleton in super-resolution microscopy**R. Hughes, A. Lopata, A. Balls, C. Tiede, D. Tomlinson, A. Curd, M. Peckham

Astbury Centre, University of Leeds, Leeds, UK

Affimers (Adhirs) are small non-antibody binding proteins (1). Based on a phytocystatin consensus sequence, they contain two loops with variable sequence, which can be screened for binding to a protein of interest. We isolated 4 different Affimers that bind to filamentous (F-) actin and assayed their ability to bind to actin *in vitro*, and to label actin in fixed and live cells. 3 of the 4 Affimers bind tightly to F-actin *in vitro*, with K_D of less than 10nM. However, only one of the four Affimers, directly labelled with an Alexa-647 dye on the unique C-terminal cysteine, labelled F-actin in fixed cells. This Affimer (Adh14) worked well in fluorescence imaging, and was particularly useful in ‘super-resolution’ 3D STORM microscopy where it outperformed phalloidin. All three Affimers that bind F-actin *in vitro* (Adh6, 14 and 24) were able to label F-actin as eGFP-fusion proteins in live cells, with subtle differences in their labelling pattern. We have now additionally isolated Affimers against tubulin, which are looking equally promising, and work well in STORM. Taken together, our data suggests that Affimers are a useful tool for imaging the cytoskeleton.

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O-151**Affimers are versatile and renewable molecular recognition reagents**C. Tiede, A. Tang, T. Taylor, D. Tomlinson, M. Mcpherson
BioScreening Technology Group, Faculty of Biological Sciences, University of Leeds, UK

We have developed a novel artificial binding protein scaffold for use as molecular recognition research tools as well as for diagnostics, imaging and therapeutic applications. Molecular recognition reagents are key tools used to study protein expression, localisation and interactions. Antibodies are the most common reagents with many being highly valuable, although some are less well characterised or suffer from stability or batch variability issues. Recently alternative binding proteins have been developed to complement antibodies. There are a number of well-known alternative binding proteins but here we report on Affimer proteins as research reagents.

Based on two randomised variable regions we have generated both one and two variable region phage display libraries of $>10^{10}$ complexity. We have selected high affinity Affimers against >400 target proteins, peptides and chemical compounds, with our normal selection process for monoclonal reagents being 2–3 weeks. Affimers can be engineered via an optional C-terminal cysteine to allow a range of modifications or molecular fusions. They have been used in ELISA, western blot analysis, pull-down assays, histochemical and immunofluorescence, super-resolution imaging, as well as to inhibit protein-protein interactions and to block protein activity. Highly specific Affimers can discriminate between highly related targets and, as they can be expressed in mammalian cells, can inhibit *in vivo* function in a highly specific manner. The binding interactions of Affimer with some target proteins have been characterised by X-ray crystallography. Affimers are also being used for the development of biosensors particularly for point-of-care antimicrobial resistance applications. Thus Affimer proteins, as with other alternative binding proteins, represent complementary affinity reagents to antibodies for a range of biochemical, biophysical and cell biology applications.

Oral Presentations

– 26. Applications in biomedical and materials science –

O-153**Force propagation in biomolecular complexes and sensory kinases**

H. E. Gaub

Physics Department, Ludwig-Maximilians Universität München, Germany

Molecular interactions are the basis of life. Forces play a crucial role in assembly and structural integrity, as well as the dynamics of all living systems. The regulation of biomolecular complexes, the maintenance of cellular structures, and even cell signaling are controlled by mechanical forces. At the molecular level, the relationships between these forces and their biological functions have become accessible through various single molecule force spectroscopy techniques developed in recent years. A deeper understanding of the physics of these relationships has emerged from the very fruitful combination of the high resolution and precision of such experiments together with the insight in structural rearrangements from all-atom Molecular Dynamics Calculations. In this talk a brief overview on the basics will be given, followed by a report on recent discoveries: The activation mechanisms of two prominent intracellular force sensors, Myosin Light Chain Kinase and Titin Kinase were elucidated. The force propagation pathways and the clamp-mechanism of catch bonds between Cohesin and Dockerins in Cellulosome complexes could be resolved.

O-155 (P-800)**Atomic force microscopy as a tool to evaluate the risk of cardiovascular diseases in patients**A. F. Guedes¹, F. A. Carvalho¹, I. Malho¹, N. Lousada², L. Sargento², N. C. Santos¹

¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; ²Unidade de Insuficiência Cardíaca, Departamento de Cardiologia, Hospital Pulido Valente, Centro Hospitalar Lisboa Norte, Lisbon, Portugal

The availability of biomarkers to evaluate the risk of cardiovascular diseases is limited. High fibrinogen levels have been identified as a relevant cardiovascular risk factor but the biological mechanisms remain unclear. Increased aggregation of erythrocytes (red blood cells) has been linked to high plasma fibrinogen concentration. Here, we show using atomic force microscopy, that the interaction between fibrinogen and erythrocytes is modified in chronic heart failure patients. Ischemic patients showed increased fibrinogen-erythrocyte binding forces compared with non-ischemic patients. Cell stiffness in both patient groups was also altered. A 12-month follow-up shows that patients with higher fibrinogen-erythrocyte binding forces initially were subsequently hospitalized more frequently. Our results show that atomic force microscopy can be a promising tool to identify patients with increased risk for cardiovascular diseases. [Guedes *et al.* (2016) *Nature Nanotechnol.*, doi:10.1038/nnano.2016].

O-154**Lego-Style Construction of Future Therapeutics From DNA**W. Shih^{1,2}

¹Harvard Medical School, USA; ²Dana-Farber Cancer Institute, USA; ³Wyss Institute at Harvard, USA

The shape of a biomolecule largely determines its function in a cell. I will discuss how we can design and self-assemble custom molecular shapes out of building blocks made of DNA, and how these miniscule devices could have a profound impact on fields ranging from molecular biophysics to therapeutics to nano-optics for decades to come.

O-156 (P-801)**Micro-structured compartment models for synthetic biology**H. Eto¹, M. Heymann¹, N. Soga², H. Noji², P. Schwill¹

¹Max-Planck-Institute of Biochemistry, Martinsried, Germany; ²Department of Applied Chemistry, University of Tokyo, Japan

Vesicle bilayer systems have been extensively employed to investigate protein-membrane interactions. When investigating peripheral membrane proteins, it often suffices to replace the bilayer by monolayer and work in water-in-oil droplets, which are more flexible and versatile in preparation. Microstructured wells coated by membrane have been used for this purpose, which, however, often pose the challenge of proper sealing. We investigate compartmentalised droplet-bilayer interfaces that encapsulate femto-litre droplet volumes under a free-standing bilayer. Based on fluoro-polymer chemistry, these microdevices can be patterned into various geometries and can facilitate high-throughput assays to analyse membrane-intercalating or interfacing proteins. We discuss the reconstitution of spatially self-organizing proteins in these isolated-volume microcompartments.

Oral Presentations

– 26. Applications in biomedical and materials science –

O-157 (P-802)**Cellular sensing platform for biomedical applications**

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¹International Centre of Biodynamics, Intr Portocalelor, Nr 1B, 060101, Bucharest, Romania; ²Faculty of Biology, University of Bucharest, Splaiul Independentei, Nr 91-95, 050095, Bucharest, Romania

High-sensitive methods that provide noninvasive real-time detection of cellular responses to pharmacological agents and biochemical compounds with therapeutic potential are needed in biomedical research. To this end, we advance a cellular platform integrating electrical impedance spectroscopy (EIS) and a model cell line (human colon cancer HT-29 cells) for investigating the effectiveness of emergent Carbonic Anhydrase Inhibitors (CAIs) (sulfonamides and sulfocoumarins) as anticancer drugs.

EIS enabled label-free, noninvasive real-time evaluation of the effect of CAIs on HT-29 cell monolayers subjected to hypoxic conditions. The results reveal specific alterations occurring within the cell layer, especially at the cell-surface level induced by the application of CAIs and hypoxic conditions. Factors like changes in cell-substrate adherence, cell number and cell-cell interactions influence the measured signal thus allowing discriminating the inhibitory capacity of the compounds directed against CA IX, a tumor specific metalloenzyme regulating extra- and intercellular pH towards tumour survival growth and metastasis. Our results indicate a powerful biosensing approach for the evaluation of CAIs potency, effective for anticancer pharmacological agents screening and design.

Oral Presentations**– 27. Protein folding and assembly –****O-158****Conditional interactions in cellular hubs**

B. B. Kragelund, K. Bugge, L. Staby, A. Prestel, N. Wickman, K. Skriver

Department of Biology, University of Copenhagen, Denmark

Disordered regions in proteins are essential cellular decision-making hot-spots and decoding of their functional roles is one of the most important open questions in biology today. Using two different folded hubs from two different organisms that each interact with many (>30) disordered partners, we are trying to understand the biological roles of protein intrinsic disorder in conditional cellular control. The ambition is to delineate mechanisms whereby cellular hubs exploit motifs in disordered protein regions to orchestrate cellular homeostasis and dynamics and how conditional phosphorylations and allostery play a role. I will present structural, biochemical and functional data of the two folded hubs and their interactions with a number of disordered partners, which show that the protein scaffold of the folded hub is deterministic in partner selection and that adaptation to the binding sites occurring either by folding-upon-binding via the formation of different structures or via maintaining disorder in the bound state, is crucial for affinity and specificity. The result originating from the two systems may provide a basis for extraction of general properties pertaining to assembly of complexes involving disordered proteins.

O-160 (P-866)**Self-organizing amyloid in bacteria**

D. E. Otzen¹, B. S. Vad¹, M. S. Dueholm², P. H. Nielsen², S. L. Rouse³, S. J. Matthews³

¹iNANO, Aarhus University, Denmark; ²Aalborg University, Denmark; ³Imperial College, UK

Functional bacterial amyloid proteins (FuBA) serve multiple purposes such as surface adhesion, biofilm formation, enhanced surface hydrophobicity, extracellular casing and cell wall strengthening. FuBA are extremely robust, often surviving boiling SDS, and are evolutionarily optimized to form amyloid structures under a wide range of condition, though often natively unfolded as monomers. FuBA systems appear to rely on two central features:

(1) **Sophisticated export and assembly systems.** Cells involve a cohort of ancillary proteins to form a FuBA export-and-assembly system in the outer membrane. Our recent structure of the export channel suggests that these ancillary proteins sculpt the folding energy landscape with a combination of nucleating proteins and “folding slides”.

(2) **Sequence design to avoid uncontrolled aggregation.** FuBAs have variable number of imperfect repeats of around 20-40 residues predicted to form hairpin structures in a monomeric structure that recapitulates the overall amyloid fold but only folds upon self-assembly. Our mechanistic studies indicate that FuBA aggregation is driven by a primary nucleation nucleation process which avoids a secondary nucleation step, unlike pathological amyloid like A-beta. This avoids an uncontrollable auto-catalytic feedback loop.

O-159**Touring the Protein Energy Landscape: the view depends on how and when you look**

S. Marqusee

Institute of Quantitative Biosciences, Dept of Molecular and Cell Biology, University of California, Berkeley, USA

Understanding the structural and dynamic information encoded in the primary sequence of a protein is one of the most fundamental challenges in modern biology. The amino acid sequence of a protein encodes more than the native three-dimensional structure; it encodes the entire energy landscape – an ensemble of conformations whose energetics and dynamics are finely tuned for folding, binding and activity. Small variations in the sequence and environment modulate this landscape and can have effects that range from undetectable to pathological. I will present our recent results probing these sequence and environmental effects using a combination of single-molecule and ensemble-based studies.

I will address a fundamental question in protein folding of whether proteins fold through one or multiple trajectories and its implications for protein folding in complex environments, and the suggestion that evolution can modulate both the rates of folding and the specific pathway.

O-161 (P-865)**Disorder-to-order transitions involved in secretion, folding and functions of a bacterial toxin**

D. P. O'Brien¹, A. Voegele¹, S. Cannella¹, D. Durand², P. Vachette², S. Brier¹, D. Ladant¹, A. Chenal¹

¹Institut Pasteur, CNRS, France; ²I2BC, CNRS, France

The adenylate cyclase toxin (CyaA) plays an essential role in the early stages of respiratory tract colonization by *Bordetella pertussis*, the causative agent of whooping cough. Once secreted, CyaA invades eukaryotic cells, leading to cell death. The cell intoxication process involves a unique mechanism of translocation of the CyaA catalytic domain directly across the plasma membrane of the target cell. In the low calcium environment of the bacterial cytosol, the C-terminal RD domain is an intrinsically disordered coil, appropriately sized for transport through the narrow secretion machinery. Upon secretion, the high calcium concentration in the extracellular milieu induces the refolding of RD, which likely acts as a scaffold to favour CyaA refolding. Due to its hydrophobic character, CyaA is known for its propensity to aggregate into multimeric forms in the absence of a chaotropic agent in vitro. We have recently showed that calcium binding and molecular confinement are critical for CyaA folding into a stable, monomeric and functional state. Overall, this data demonstrates the adaptation of bacterial RTX toxins to the diverse array of calcium concentrations encountered in the successive environments during the cell intoxication process.

Oral Presentations*– 27. Protein folding and assembly –***O-162 (P-864)****Dual function of the trigger factor chaperone in nascent protein folding**K. Liu, K. Maciuba, C. M. Kaiser

Johns Hopkins University, Department of Biology, USA

Large proteins often require help from molecular chaperones to fold productively, even before the ribosome has finished their synthesis. The mechanisms of chaperone function remain poorly understood. Using optical tweezers to study the folding of nascent elongation factor G (EF-G), a model multi-domain protein, we find that the N-terminal G-domain folds robustly on the ribosome. The following domain II, in contrast, fails to fold efficiently. Strikingly, interactions with the unfolded domain II convert the natively folded G domain to a non-native state that readily unfolds. The two unfolded domains subsequently form misfolded states. Both the conversion of natively folded domains and non-productive interactions among unfolded domains are efficiently prevented by the nascent chain-binding chaperone trigger factor. Thus, our single-molecule measurements reveal an unexpected role for the chaperone: It protects already folded domains against denaturing interactions with parts of the nascent polypeptide that are not yet folded. Previous studies had implicated trigger factor in guiding the folding of individual domains, but interactions among domains had been neglected. Avoiding early folding defects is crucial, since they can propagate and result in misfolding of the entire protein.

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Oral Presentations**– 28. Morphogenesis and development –****O-163****The mechanical control of nervous system development**

K. Franze

Department of Physiology, Development and Neuroscience, University of Cambridge, UK

During development and pathological processes, cells in the central nervous system (CNS) are highly motile. Despite the fact that cell motion is driven by forces, our current understanding of the mechanical interactions between CNS cells and their environment is very limited. We here investigated the mechanical control of neuronal growth in the developing brain. *In vitro*, growth and migration velocities, directionality, cellular forces as well as neuronal fasciculation and maturation all significantly depended on substrate stiffness. Moreover, when grown on substrates incorporating linear stiffness gradients, axon bundles turned towards soft substrates while glial cells migrated in the opposite direction. *In vivo* atomic force microscopy measurements revealed stiffness gradients in developing brain tissue, which axons followed as well towards soft. Interfering with brain stiffness and mechanosensitive ion channels *in vivo* both led to similar aberrant neuronal growth patterns with reduced fasciculation and pathfinding errors. Substrate stiffness not only directly impacted neuronal growth but also indirectly by regulating neuronal responses to chemical guidance cues, strongly suggesting that neuronal growth is not only controlled by chemical signals – as it is currently assumed – but also by the tissue's local mechanical properties.

O-165**Cell morphogenesis across scales, from molecular processes to cell surface mechanics**

E. Paluch

MRC LMCB, University College London, UK

The shape of animal cells is primarily determined by the cellular cortex, a thin network of actin filaments and myosin motors bound to the plasma membrane. We investigate how the mechanical properties of the cell surface arise from the microscopic organisation of the cortical network, and how changes in these properties drive cell deformation. We have developed methods to investigate the nanoscale architecture of the cortex and are exploring how the organisation of actin filaments controls network mechanics. We also investigate how the spatial distribution of motor proteins in the cortex modulate cortical tension. Using a combination of cell biology experiments, quantitative imaging and theory, we aim to understand cell surface tension generation and the control of cell shape across scales.

O-164**Cell wall architecture and the development of cell shape in *S. aureus* and *E. coli***J. K. Hobbs, J. M. Burns, R. D. Turner, R. G. Bailey, S. Kumar, R. K. Tank, N. Mullin, S. J. Foster
University of Sheffield, UK

Bacterial shape is largely determined by the architecture of the peptidoglycan cell wall. During cell growth and division the wall undergoes a process of both growth and remodelling to ensure that shape is preserved from mother to daughter cells. Here we have studied the interplay between molecular level peptidoglycan architecture, mechanical properties and shape throughout the cell cycle using atomic force microscopy. In *Staphylococcus aureus*, despite its primarily spherical shape, we observe an evolution of wall stiffness that corresponds to enzymatic activity, revealing a growth mechanism that depends on peptidoglycan degradation. Molecular resolution imaging reveals a corresponding change in architecture from tightly packed concentric rings to a random and highly porous mesh more akin to a gel. In *Escherichia coli* we have been able to clearly image the molecular organisation of the near two dimensional peptidoglycan wall, revealing unexpected variations in ordering and complexity. Correlative work with super-resolution optical microscopy (STORMForce) will also be presented as a route towards adding chemical detail to morphological and mechanical information from AFM.

O-166 (P-921)**Decoding temporal interpretation of the morphogen Bicoid in the early *Drosophila* embryo**A. Huang¹, C. Amourda¹, S. Zhang¹, N. S. Tolwinski^{2,3}, T. E. Saunders^{1,3}

¹Mechanobiology Institute, National University of Singapore, Singapore; ²Division of Science, Yale-NUS College, Singapore; ³Department of Biological Sciences, National University of Singapore, Singapore

Morphogen gradients provide essential spatial information during development. Not only the local concentration but also duration of morphogen exposure is critical for correct cell fate decisions. Yet, how and when cells temporally integrate signals from a morphogen remains unclear. Here, we use optogenetic manipulation to switch off Bicoid-dependent transcription in the early *Drosophila* embryo with high temporal resolution, allowing time-specific and reversible manipulation of morphogen signalling. We find that Bicoid transcriptional activity is dispensable for embryonic viability in the first hour after fertilization, but persistently required throughout the rest of the blastoderm stage. Short interruptions of Bicoid activity alter the most anterior cell fate decisions, while prolonged inactivation expands patterning defects from anterior to posterior. Such anterior susceptibility correlates with high reliance of anterior gap gene expression on Bicoid. Therefore, cell fates exposed to higher Bicoid concentration require input for longer duration, demonstrating a previously unknown aspect of morphogen decoding.

Oral Presentations**– 28. Morphogenesis and development –****O-167 (P-920)****The physical basis of coordinated tissue spreading in zebrafish gastrulation**H. Morita^{1,2}, S. Grigolon³, M. Bock⁴, S. F. G. Krens², G. Salbreux³, C.-P. Heisenberg²¹Graduate School of Medical Sciences, University of Yamanashi, Japan; ²IST, Austria; ³The Francis Crick Institute, UK; ⁴Max Planck Institute for the Physics of Complex Systems, Germany

Embryo morphogenesis relies on highly coordinated movements of different tissues. Yet, remarkably little is known about how tissues coordinate their movements to shape the embryo. Tissue coordination becomes first apparent during one of the main stages of vertebrate development, namely gastrulation. In zebrafish embryos, the onset of gastrulation is characterised by the spreading of a tissue, the blastoderm, over a yolk sac and this process is commonly thought to be mediated by radial cell intercalations. These movements simultaneously narrow the tissue along its height (radial extent) and expand it along its plane. Yet, whether radial cell intercalations drive tissue spreading or represent the response of the tissue to exogenous spreading forces remains unclear. In this talk, we use a combination of active gel theory and experiments (by Dr. Hitoshi Morita) to dissect the fundamental force-generating processes underlying the initial spreading of the blastoderm over the yolk cell at early zebrafish gastrulation. Unexpectedly, we found that active radial cell intercalations are dispensable for blastoderm spreading per se and that, instead, this process is driven by epithelial surface cells autonomously reducing their surface tension and thus actively expanding.

O-168 (P-919)**Epithelium adaptation to external curvature *in vitro***C. Tomba, F. Maechler, A. Trushko, I. Di Meglio, A. Roux
University of Geneva, Department of Biochemistry, Switzerland

Deformation of flat epithelia into a given shape is specific to each organ and its function in the organism. For instance, during the gut formation, an initially smooth gut tube is formed and then intestinal villification can take origin by muscle constriction (Shyer et al., Science, 2013). Once the villi shape is established, it is maintained throughout of life. Therefore, gut cells have to function in regions of different curvature. Despite of the growing evidence of the interplay between external forces, mechanotransduction and organ morphology, little is known about cell adaptation to external geometrical constraints. For this study, we have developed two complementary techniques to control epithelium curvature and to investigate its possible role in cell growth and organisation. In the first case we induce an initially flat epithelium on PDMS substrates to deform into a given curvature; in the second case we investigate epithelial cell growth encapsulated in alginate tubes. These systems have the advantage to provide simple tools to control the physical cell environment and to isolate the effects of its properties on cell growth. In particular, our researches focus on quantitative studies of epithelial monolayer adaptation, e.g. in terms of cell shape and proliferation.

Oral Presentations**– 29. Optogenetics and neural systems –****O-169*****In vivo* two-photon optogenetics with sculpted light**

V. Emiliani

CNRS and University Paris Descartes, France

Optogenetics has revolutionized neuroscience by enabling remote activation or inhibition of specific populations of neurons in intact brain preparations through genetically targeted light sensitive channels and pump. Nevertheless, studying the role of individual neurons within neuronal circuits is still a challenge and requires joint progresses in opsin engineering and light sculpting methods. Here we show that computer generated holography using an amplified pulsed laser combined with high light sensitive opsins [1, 2] enable precise in vitro and in vivo control of neuronal firing in mouse brain with millisecond temporal precision, single cell resolution and unprecedented low illumination level.

We also show a new optical system enabling remote axial displacement of temporally focused holographic patterns, as well as generation of multiple temporally focused holographic targets occupying separate axial planes opsins [3]. We demonstrate the capabilities of the system to probe the functional connectivity between the bipolar cell layer and the ganglion cell layer in the mice retina.

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O-171**Hearing the light: optogenetic stimulation of the auditory pathway**T. Moser^{1,2}, C. O. Göttingen^{1,3}

¹Institute for Auditory Neuroscience, University of Göttingen Medical Center, Göttingen, Germany; ²Auditory Neuroscience and Optogenetics Laboratory, German Primate Center, Göttingen, Germany; ³MPI for Experimental Medicine, Göttingen, Germany

When hearing fails, speech comprehension can be restored by auditory prostheses such as the cochlear implant (CI). We aim to improve frequency and intensity resolution of CI coding by establishing spatially confined optical stimulation of spiral ganglion neurons (SGNs). We have established optogenetic stimulation of the auditory pathway in rodents using virus-mediated expression of channelrhodopsins to render SGNs light-sensitive. Optogenetic stimulation of spiral ganglion neurons using blue and orange light activated the auditory pathway, as demonstrated by recordings of single neuron and neuronal population responses at various stages of the auditory system. Fast red-shifted opsins enabled firing of individual SGNs at near physiological rates (hundreds per second). Towards characterizing the percept induced by cochlear optogenetics we studied activation of neurons in primary auditory cortex and performed behavioral analysis using the blue channelrhodopsin CatCh. Towards the development of optical CIs we collaborate on microscale light emitting diodes and demonstrate the functionality of first multichannel optical CIs. In summary, optogenetic stimulation of the auditory nerve is feasible and bears great potential for future application in research and hearing restoration.

O-170**Closed-loop real-time all-optical interrogation of neural circuits *in vivo***

Z. Zhang, L. E. Russell, A. M. Packer, O. M. Gauld, M. Hausser

Wolfson Institute for Biomedical Research, University College London, UK

Neural activity patterns in the intact brain are rapidly evolving and highly variable from trial to trial. It is therefore crucial to be able to read out and interfere with these patterns in real time. Such closed-loop interventions would provide direct causal links between specific activity patterns and behaviour. I will describe a closed-loop all-optical strategy for dynamically controlling neuronal activity patterns in awake behaving mice. This approach involves rapidly tailoring and delivering two-photon optogenetic stimulation based on read-out of activity using simultaneous two-photon imaging of the same neural population. This closed-loop feedback control can be used to clamp neural activity at pre-defined levels, yoke together activity in neighbouring neurons, and boost weak sensory-evoked responses. This approach allows the rate and timing of activity patterns in neural circuits to be flexibly manipulated ‘on the fly’ during behavior.

O-172 (P-928)**A novel neurophotonic approach to study neural networks in vitro**

W. Afshar Saber, F. M. Gasparoli, M. G. Dirks, F. J. Gunn-Moore, M. Antkowiak

University of St Andrews - School of Medicine, UK

Optogenetics is an emerging field that benefits from the synergy of optical and genetic techniques [1]. It allows non-invasive activation or inhibition of specific neurons using only light and enables repetitive interrogations of the same cells. It has been combined with purely optical readout of neural activity (using fluorescent voltage reporters) in a technique called Optical Electrophysiology [2] but this requires extreme microscopy which limits its use in pre-clinical and drug screening applications. To overcome these limitations, we have recently developed advanced protocols in which calcium imaging can be used to quantify network dynamics *in vitro* using a genetically encoded calcium reporter (manuscript in preparation). This is a novel quantitative use of neurophotonic to potentially understand human cognition, investigate how diseased neurons communicate thus enabling functional *in vitro* high throughput screening and testing of dementia drug candidates.

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Oral Presentations**– 29. Optogenetics and neural systems –****O-173 (P-929)****Signaling states of short LOV proteins and their implications for construction of optogenetic tools**R. Batra-Safferling¹, J. Granzin¹, V. Arinkin¹, K. Röllén¹, U. Krauss², K.-E. Jaeger², D. Willbold¹¹Institute of Complex Systems, ICS-6: Structural Biochemistry, Forschungszentrum Jülich, Germany; ²Institut für Molekulare Enzymtechnologie, Heinrich-Heine-Universität Düsseldorf, Forschungszentrum Jülich, Germany; ³Institut für Experimentalphysik, Freie Universität Berlin, Germany

Unique features of Light-Oxygen-Voltage (LOV) proteins like relatively small size (~12–19 kDa), inherent modularity, highly-tunable photocycle and oxygen-independent fluorescence have lately been exploited for the generation of optogenetic tools.

Here, we will provide insights of the light-activated signaling mechanism in a short LOV protein PpSB1-LOV from a comparison of crystal structures obtained in the dark- and fully light-adapted states. Major structural differences involve a~11Å movement of the C terminus in helix Jα, disruption of the dimer interface, and a~29° rotation of chain-B relative to chain-A in the dark state. The activation mechanism supports a rotary switch mechanism and provides insights into the signal propagation mechanism in naturally existing and artificial LOV-based, two-component systems.

Second, we will present the first apo-state crystal structure of a PpSB1-LOV homologue- W619_L1 (89% sequence identity). The apo protein binds both natural and structurally modified flavin chromophores, revealing remarkably different photophysical and photochemical properties. These results imply application of these selective variants as novel optical tools as they offer advantages such as no adduct state formation, and a broader choice of wavelengths for in vitro studies.

O-174 (P-930)**Orchestrating cells on a chip employing standing surface acoustic waves towards neural networks**M. S. Brugger¹, S. Grundeen², L. Theogarajan², A. Wixforth^{1,3}, C. Westerhausen^{1,3}¹Chair for Experimental Physics 1, University of Augsburg, 86159 Augsburg, Germany; ²Department of Electrical and Computer Engineering, UCSB, CA, 93106, USA; ³Nanosystems Initiative Munich, 80799 Munich, Germany

We propose the implementation of a new lab-on-a-chip based system for the controlled growth and formation of complex neural networks on a semiconductor chip. By combining microfluidic techniques with surface acoustic waves (SAW), we can create and stimulate simple life-on-a-chip systems. For this purpose, we constructed a chip consisting of a piezoelectric LiNbO₃ substrate and four interdigital transducers for the excitation of SAW to from standing waves with according nodes and antinodes in a checkerboard pattern. The anticipated formation of the pressure node lattice has been visualized using atomic force microscopy. By adding a PDMS-microchannel, this equidistant and regular patterning lattice allows us to simultaneously control the position of objects in a liquid environment in space and time. The possibility and accuracy to pattern cell-sized single objects on these chips were validated by patterning small beads of different sizes. Ensuring the conditions for cell growth, we successfully demonstrate single cell alignment, their adhesion and growth within the well-defined pressure nodes on the chip. Finally, we verified the biocompatibility of SAW for primary neural cells. This gives us confidence that it will allow us to apply our technique to single neurons in the future.

Oral Presentations

– 30. Ionic liquids meet biomolecules –

O-175

Ionic liquids as biocompatible co-solvents for the stability of biomolecules

V. Pannur

University of Delhi, India

We have investigated the biomolecular interactions and related associated structural changes of α -chymotrypsin (CT) with new ILs by using several biophysical techniques including circular dichroism (CD) and fluorescence. The ILs studied in the present study includes diethylammonium dihydrogen phosphate $[(CH_3CH_2)_2NH][H_2PO_4]$ (DEAP), diethylammonium hydrogen sulfate $[(CH_3CH_2)_2NH][HSO_4]$ (DEAS), triethylammonium dihydrogen phosphate $[(CH_3CH_2)_3NH][H_2PO_4]$ (TEAP) and triethylammonium hydrogen sulfate $[(CH_3CH_2)_3NH][HSO_4]$ (TEAS). We observed that all ILs have dominant contribution to the stabilization of the native structure of the CT. Furthermore, the results reveal that phosphate anions of ILs are strong stabilizers and acted as effective refolding enhancers for thermally denatured enzyme structure, whereas the enzyme was not refolded in the sulfate anions of ILs. These findings suggest a new generation of enzyme stabilizers that can be applied to other protein folding studies and biological systems.

O-177

Experimental visualization of interactions between ionic liquids, water and biomolecules

V. P. Ananikov

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IOLIOICS is a rapidly growing area of research dealing with the studies of ions in liquids and stipulated with fundamental differences of ionic interactions [1]. Recently, we have reported experimental observation of dynamic interactions directly in the ionic liquids using electron microscopy [2]. Self-organization of water in ionic liquids led to the formation of a variety of morphologies, including droplets, aggregates and networks [2]. Detailed investigation of this system provided valuable driving force for improving utility of biomass conversion in organic synthesis [3]. Using electron microscopy it was possible to visualize the process involving biomolecules in ionic liquids [2]. Carbohydrates and peptides were studied with NMR spectroscopy and mass-spectrometry to develop practical applications for efficient extraction [4]. The key role of ionic interactions was also explored in different drug development platforms [5].

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O-176

Ionic liquids vs biomembranes: a neutron scattering, atomic force microscopy and computational study

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The interaction between ionic liquids (ILs) and biomolecules is an emerging area of research. Among biological structures, model biomembranes such as phospholipid bilayers have been the first to be investigated in relation with ILs. The recognised affinity of ILs for phospholipid bilayers, first revealed in a short series of pioneering experiments and later confirmed by computer simulations and by neutron scattering experiments, opens a vast new playground to investigate the interaction of IL with paradigmatic biological structures. We will show that this far-reaching goal can be achieved by using neutron scattering as a structure- and dynamics-sensitive technique, and by resorting to atomic force microscopy (AFM) and molecular dynamics simulations. We will present recent experimental results on the microscopic mechanisms that allow ILs to penetrate lipid bilayers. Whereas neutron scattering allows to determine the characteristic time of the absorption of the single cations in the lipid region, which is of order of nanoseconds, AFM shows that cations penetrates first into the bilayer defects by following a power law distribution ($\beta=1.2$). Moreover, AFM allows to probe the mechanical properties of the lipid bilayers doped with ILs, which result more rigid than the net ones.

O-178

Liquid-liquid phase separation of highly charged hen egg white lysozyme and heparin at pH = 2.0

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Amyloid describes a special type of protein structural state that is accessible to many, if not all proteins, upon their partial unfolding and aggregation (Hall and Edsles, 2012). Amyloid manifests as a nano- to micrometre filamentous aggregate (Hall et al. 2015; Hall 2012). A number of positively and negatively charged polymers have been shown to facilitate the conversion of oppositely charged proteins into the amyloid form. Heparin, a poly-sulfated carbohydrate polymer predominantly composed of the disaccharide monomeric unit 2-O-sulfated iduronic acid and 6-O-sulfated, N-sulfated glucosamine [IdoA(2S)-GlcNS(6S)] has the greatest negative charge density of any naturally occurring biopolymer. In this paper we use turbidity assays (Hall et al. 2016) and size-exclusion chromatography (Hall and Huang, 2012) to examine the mechanism of the initial liquid-liquid phase separation between heparin and hen egg white lysozyme at low pH, a solution environment in which all sulfate groups on the low molecular weight heparin are negatively charged and the lysozyme carries a net positive charge. We discuss the highly charged reaction environment in the context of recent developments in room temperature ionic-liquids (Benedetto and Ballone, 2015).

Oral Presentations**– 30. Ionic liquids meet biomolecules –****O-179 (P-939)****Probing interactions of cellulose, lignin and ionic liquids towards enabling sustainability**

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Carbon-rich fossil fuels provide majority of the energy consumed in the world. The high-energy content of liquid hydrocarbon fuels makes them the preferred energy source for all modes of transportation. However, non-renewable, fossil fuel based society is not sustainable. This has spurred research into alternative, non-fossil energy sources and among the options, biomass has the potential to provide a high-energy-content transportation fuel. Plant-derived biomass contains biological macromolecules like cellulose, hemicellulose and lignin. Ionic liquids (ILs) based biomass conversion technologies have recently gain a great deal of attention as a green option that could enable carbon-efficient conversion. However, the development of cost-effective processes to transform cellulose and lignin in biomass into fuels is hampered by poor understanding of fundamental biophysical processes at play and necessitate application of advanced experimental and theoretical approaches to gain a better understanding of these interactions. I will discuss both computational and experimental approaches we have been using to gain a better understanding of ILs interactions with cellulose and lignin and how our effort is enabling a whole new class of renewable ILs for bio-based economy and sustainability.

O-181 (P-941)**Interaction of imidazolium-based ionic liquids with soft supported lipid membrane**G. Bhattacharya¹, R. P. Giri², H. Saxena¹, V. V. Agrawal³, A. Gupta¹, M. K. Mukhopadhyay², S. K. Ghosh¹¹School of Natural Sciences, Shiv Nadar University, NH-91, Tehsil Dadri, G. B. Nagar, Uttar Pradesh 201314, India;²Saha Institute of Nuclear Physics, Bidhannagar, Kolkata 700064, India; ³National Physical Laboratory, Dr. K S Krishnan Marg, New Delhi 110012, India

Ionic liquids (ILs) are known to show antimicrobial activity and are observed to be toxic to some microorganisms. To understand the molecular mechanism of these activities, the interactions of imidazolium-based ILs with model biomembranes have been investigated. The pressure area- isotherm of self-assembled monolayer formed by cell membrane mimicking lipid molecules is found to be altered due to interaction with ILs. As a result, the in-plane elasticity of the monolayer is reduced. The self-assembled structure of lipid bilayer on a polymer support is perturbed as evident from x-ray reflectivity (XRR) studies. Due to the IL-membrane interaction, the bilayer thickness reduces considerably and the corresponding electron density of the layer is increased (1). The nature of the interaction not only depends on the hydrophobicity of ILs but also on the electrostatics of the system. This study provides a molecular description of IL-membrane interaction.

References

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O-180 (P-940)**Biophysical and biological activities of imidazolium-based lipid analogues**D. Wang¹, A. Rühling², P. Drücker¹, D. Grill³, F. Glorius², H.-J. Galla¹¹Institute of Biochemistry, University of Muenster,, Germany; ²Organic Chemistry Institute, University of Muenster, Germany; ³Institute of Medical Biochemistry, Center for Molecular Biology of Inflammation, University of Muenster,, Germany

Tailor-made ionic liquids based on imidazolium salts have attracted our attention due to their extraordinary properties and versatile functionality. Here we report the intriguing ability of newly synthesized 1,3-dialkylimidazolium compounds to interact with and to stabilize lipid membranes. The introduction of alkyl chains at the imidazolium backbone in the 4,5-position of N-heterocyclic carbene salts leads to a high structural similarity to the hydrophobic part of natural lipids. Their interaction with cellular membranes resulted in remarkable properties such as enhanced cytotoxicity and antitumor activity, which was interpreted by surfactant monolayer models in vitro. Membrane hydration properties and domain fluidization were analyzed by fluorescence in lipid bilayers resembling living cells. Membrane binding and insertion was analyzed via a quartz crystal microbalance and confocal laser scanning microscopy. It is shown that short-chain 4,5-dialkylimidazolium salts with a bulky head group are able to disintegrate membranes. Long-chain imidazolium salts form bilayer membrane vesicles spontaneously and autonomously without the addition of other lipids.

Oral Presentations

– 31. Imaging molecules of life –

O-182

Electron cryomicroscopy of rotary ATPases

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Our group uses cryo-EM to study the structures of rotary ATPases and related macromolecular machines. We also work to develop new methods for cryo-EM to facilitate these studies. Ion-translocating rotary ATPases serve either as adenosine triphosphate (ATP) synthases, using energy from a transmembrane ion motive force to create the cell's supply of ATP, or as transmembrane ion pumps that are powered by ATP hydrolysis. The members of this family of enzymes each contain two rotary motors: one that couples ion translocation to rotation and one that couples rotation to ATP synthesis or hydrolysis. Our recent studies have not only illuminated the structures of these fascinating molecular motors at unprecedented resolution, but have also started to uncover their dynamics through computational isolation of the different conformations of the enzymes that exist simultaneously in solution. This lecture will describe some of the tools we have helped to develop, the latest structures we have determined for the mitochondrial ATP synthase and proton pumping V-ATPase, and what we have learned about how these enzymes function and how they interact with molecules that affect their activities.

O-184 (P-952)

Peptide directed synthesis of continuous DNA nanowires for analysis of large DNA molecules with SEM

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¹Sungkyunkwan University, South Korea; ²Sogang University, South Korea

Being an important genetic molecule with programmable functionality, DNA is actively studied in diverse areas in cutting-edge science and technology. Although numerous studies have reported the visualization of DNA molecules, most studies were carried out with transmission electron microscopes, atomic force microscopes, or fluorescence microscopes, which are actually not desirable for whole analysis of micro/millimeter sized DNA molecules with nanometer sized features.

In this work, we developed a novel approach to synthesize smooth and continuous DNA nanowires applicable for analysis of large DNA molecules by SEM. By introducing thiol-tagged DNA binding peptides, we could densely introduce thiol groups into native DNA molecules. This allowed us to immobilize DNA molecules on conductive gold substrates and densely anchor gold nanoparticles onto DNA backbone. Subsequently, we could use these gold nanoparticles as seeds to guide smooth and continuous DNA templated metallization. Using our method, we not only could image smooth and uniform structures of long DNA (λ -DNA, 48.5 kb), its dimer (97 kb), and trimer (145.5 kb), but also observe entangled 3 dimensional images of DNA using SEM, which are very difficult to be achieved with other analytical techniques.

O-183

Antigens on the move – Structure and mechanism of translocation machineries in adaptive immunity

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Biocenter, Goethe University Frankfurt, Germany

Recognition and elimination of virally or malignantly transformed cells are fundamental tasks of the adaptive immune system. For immune surveillance, the metastable cellular proteome is displayed as broken bits (peptides) on major histocompatibility (MHC) class I molecules to cytotoxic T-lymphocytes. Our knowledge about the track from the equivocal protein to the presentation of peptides has greatly expanded, leading to a quite elaborate understanding of the MHC I antigen processing pathway. I will report on the structure, energetics, and conformational dynamics of heterodimeric ABC transporters, leading to a strictly unidirectional movement of antigens. Using an integrative approach, e.g. cryo-EM, X-ray, the contribution of individual proteins as well as the architecture of the peptide-loading complex will be discussed, including mechanisms of viral immune evasion. References:

Nöll *et al.* (2017) Structure and mechanistic basis of a functional homolog of the antigen transporter TAP. *PNAS* **114**, 438

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O-185 (P-953)

Single-molecule dynein, kinesin and IFT particle dynamics at the *C. elegans* ciliary tip

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LaserLaB and Department of Physics and Astronomy, Vrije Universiteit, Amsterdam, Netherlands

Cilia are polar, microtubule-based cellular sensing hubs that rely on intraflagellar transport (IFT) for their development, maintenance and function. IFT trains, consisting of the IFT-A and IFT-B protein complexes and cargo, are co-operatively driven from ciliary base to tip by kinesin-2 motors. At the tip, trains turn around and are transported back to the base by IFT dynein. The mechanism of this turnaround at the tip has remained elusive. Here, we employ single-molecule fluorescence microscopy of IFT components in the tips of phasmid cilia of living *C. elegans*. Analysis of the trajectories reveals distinct turnaround behavior of different IFT components: while the motor proteins and IFT-A particle subcomplexes mostly turn around immediately, IFT-B particle subcomplexes show substantial pauses, lasting around 3 seconds. Our data provides the first *in vivo* single-molecule quantification of IFT tip turnarounds, providing new, comprehensive insights into this vital part of bidirectional transport in sensory cilia.

Oral Presentations*– 31. Imaging molecules of life –***O-186 (P-954)****Near infrared fluorescent nanosensors for chemical imaging of chemical communication between cells**

S. Kruss

Institute of Physical Chemistry, Göttingen University, Germany

Nanomaterials are versatile building blocks for fluorescent biosensors. We use carbon nanomaterials such as semiconducting single-walled carbon nanotubes (SWCNTs) as building blocks for such sensors. SWCNTs fluoresce in the near infrared (nIR) and their optoelectronic properties are very sensitive to changes in the chemical environment.

In general, a sensor requires a recognition unit but well-known motifs such as antibodies are often too large to achieve good sensitivity. Therefore we followed a new approach and created recognition motifs by letting smaller macromolecules such as DNA and DNA-peptide constructs directly adsorb and fold on the SWCNT surface. Using this concept we created different organic phases around SWCNTs and show that the best candidates are able to recognize certain biomolecules such as neurotransmitters or bacterial motifs.

Arrays of these sensors can be used for chemical imaging of small molecules. In order to predict the spatial and temporal resolution of this approach we additionally developed a Monte-Carlo based stochastic kinetic simulation and found that the rate constants play a decisive role. Finally, we show how such sensors can be used for nIR chemical imaging of neurotransmitter release by neuronal cells and pathogen detection.

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Oral Presentations**– 32. Receptors and signalling –****O-187****Optogenetics: basics, applications and chances**E. Bamberg¹, T. Moser², V. Gordeliy³

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Microbial Rhodopsins are widely used in these days as optogenetic tools in neuro and cell biology. We were able to show that rhodopsins from the unicellular alga *Chlamydomonas reinhardtii* with the 7 transmembrane helix motif act as light-gated ion channels (channelrhodopsins, ChR1, ChR2). Together with the light driven Cl⁻ pump Halorhodopsin ChR2 is used for the non-invasive manipulation of excitable cells and living animals by light with high temporal resolution and more important with extremely high spatial resolution. The basic functional mechanism and structural description of this unusual class of ion channels is given (electrophysiology, noise analysis, flash photolysis 2D and 3D crystallography with a high resolution structure of the WT and a mutant). Structure based engineered new tools are presented, which allow high frequency response of different neuronal cells. Their application with a biomedical perspective of an optogenetic cochlea implant is discussed.

O-189**Imaging the early events of T-cell triggering**

D. Klenerman

University of Cambridge, UK

The triggering of the T-cell receptor (TCR) by peptides bound to the major histocompatibility complex is a key event in initiating T-cell signalling and mounting an adaptive immune response. This process is both highly specific and highly sensitive, however it is not understood how this is achieved at the molecular level. To address this problem it is essential to be able to firstly determine the resting state of the TCR and the other key signalling molecules and then to follow the changes that occur during triggering. I will firstly present new quantitative methods to measure the TCR resting state, unperturbed by the surface, and then show how single molecule fluorescence imaging can be used to follow triggering on glass surfaces in the absence of ligands and at cell-cell contacts.

O-188**Tethered signalling reactions on immune receptors**J. Goyette¹, C. Solis Salas¹, N. Coker Gordon¹, M. Bridge¹, S. Isaacson², J. Allard³, O. Dushek¹¹University of Oxford, UK; ²Boston University, USA;³University of California, Irvine, USA

Tethered enzymatic reactions are ubiquitous in signalling networks but are poorly understood. Here, a novel mathematical analysis is established for tethered signalling reactions in surface plasmon resonance (SPR). Applying the method to the phosphatase SHP-1 interacting with a phosphorylated tether corresponding to an immune receptor cytoplasmic tail provides 5 biophysical/biochemical constants from a single SPR experiment: two binding rates, two catalytic rates, and a reach parameter. Tether binding increased the activity of SHP-1 by 900-fold through a binding-induced allosteric activation (20-fold) and a more significant increase in local substrate concentration (45-fold). The reach parameter indicates that this local substrate concentration is exquisitely sensitive to receptor clustering. We further show that truncation of the tether leads not only to a lower reach but also to lower binding and catalysis. The work establishes a new framework for studying tethered signalling processes and highlights the tether as a control parameter in clustered signalling.

O-190 (P-980)**Impact of membrane lipid composition on Dopamine D2 receptor activation**M.-L. Jobin^{1,2}, V. de Smedt-Peyrusse², J. A. Javitch³, J.-L. Banères⁴, T. Durroux⁵, I. D. Alves¹, P. Trifilieff²

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The dopamine D2 receptor (D2R) is a 7-transmembrane receptor that strongly interacts with membrane lipids, however, the implication of the membrane lipid environment on D2R properties has been overlooked. Yet, the brain is highly enriched in lipids and in polyunsaturated fatty acids (PUFAs) in particular, which are known to impact the properties of membranes as well as the activity of transmembrane proteins. Recent *in vivo* data obtained in the lab suggest that membrane PUFA content impacts the functionality and signaling properties of the D2R. Our project aim was to unravel the impact of membrane lipid composition on D2R conformation and pharmacological properties through biophysical studies in both cell membrane fragments and membrane models systems. To this aim, we have performed *in cellulo* and *in vitro* studies using fluorescence anisotropy and plasmon waveguide resonance (PWR) on cells enriched with specific PUFAs and in lipid reconstituted model systems of controlled lipid composition. Overall the data indicates that n-3 and n-6 PUFA enhance both agonist and antagonist affinity for D2R. The results could have a significant impact in the development of novel therapeutic strategies for psychiatric disorders in which the D2R plays a key role.

Oral Presentations**– 32. Receptors and signalling –****O-191 (P-981)****Unraveling the mystery of the seemingly too short linker in bivalent ligands of opioid receptors**K. Pluhackova¹, R. A. Böckmann¹, P. Kolb²¹Computational Biology, Department of Biology, Friedrich-Alexander University of Erlangen-Nürnberg, Germany; ²Department of Pharmaceutical Chemistry, Philipps-Universität Marburg, Germany

Linking G-protein coupled receptor (GPCR) dimers by bivalent ligands (BLs) is an elegant method for studying and altering biological activities of GPCRs [Shonberg et al, *ChemMedChem* 2011]. BLs with the largest pharmacologic impact target an opioid heterodimer consisting of inactive receptor δ (DOR) and active receptor μ (MOR*). Thereby, tolerance and dependence caused by MOR agonists (e.g. morphium) is significantly reduced [Daniels et al, *PNAS USA* 2005]. A common opinion is that the individual ligands are buried in the orthosteric pockets of the receptors while the linker is located in the extracellular space. However, such linkers would need to be 4–5 nm long [Glass et al, *TrendsPharmacolSci* 2016] which contradicts the functionality of multiple known BLs with linkers shorter than 2.5 nm.

Here by multiscaling molecular dynamics simulations, we studied the required linker length connecting a μ agonist and a δ antagonist [Harvey et al, *ACS MedChemLett* 2012] in different DOR/MOR* dimers. In the most potent dimers the ligands were connected by a 2 nm-long linker. The stability of the ligand in the subsequent atomistic simulation confirms that BLs with short linkers (~2 nm) can bind two GPCRs simultaneously. Thereby, the linker passes between two helices directly to the next receptor.

O-192 (P-982)**Proton-induced conformational switching in GPCRs is tailored to the membrane interface**A. Sandoval², S. Eichler¹, S. Madathil¹, P. Reeves³, R. A. Böckmann², K. Fahmy¹¹Helmholtz-Zentrum Dresden - Rossendorf, Germany;²University of Erlangen, Germany; ³University of Essex, UK

Ionic and H-bond interactions between the cytosolic ends of transmembrane helices TM3 and TM6 of class-A (rhodopsin-like) G protein-coupled receptors (GPCRs) stabilize the inactive receptor conformation. In the photoreceptor rhodopsin, proton uptake at Glu134 in the conserved E(D)RY motif breaks this "ionic lock" leading to receptor activation. Our MD calculations on membrane-embedded TM3 peptides show that protonation of the conserved glutamic acid alters the side chain rotamer preference and stabilizes the C-terminal helical structure. The altered peptide topology at the membrane interface rises of the side chain pKa (> 6) and lowers the polarity around the TM3 C-term as revealed also by fluorescence spectroscopy. The effects were not seen with an amide. Also time-resolved FTIR spectroscopy showed different kinetics for lipid ester carbonyl hydration around TM3, suggesting that the carboxyl is linked to a more extended H-bond network than an amide at the same position. The same was seen in DOPC-reconstituted opsin mutants (carrying a Glu134 or Gln134). Thus, the E(D)RY motif is a proton-regulated hydrated membrane microdomain. It acts as a proton switch through reorganization of the water H-bond network at the membrane interface.

Oral Presentations

– 33. Membrane-active peptides –

O-193

Membrane structural transitions during peptide binding: Implications for drug design

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Monash University, Australia

Biomolecular-membrane interactions play a critical role in the regulation of many important biological processes such as protein trafficking, cellular signalling and ion channel formation. Peptide/protein-membrane interactions can also destabilise and damage the membrane which can lead to cell death. Characterisation of the molecular details of these binding-mediated membrane destabilisation processes is therefore central to understanding cellular events such as antimicrobial action, membrane-mediated amyloid aggregation, and apoptotic protein induced mitochondrial membrane permeabilization. Optical biosensors have provided a unique approach to characterising membrane interactions allowing quantitation of binding events and new insight into the kinetic mechanism of these interactions. We have developed dual polarisation interferometry (DPI) to allow biophysical analysis of membrane structure changes. DPI allows the real-time measurement of bilayer structure changes during peptide binding to define the mechanisms of bilayer disturbance between different classes of peptides and proteins. The combination of DPI with other biophysical techniques now opens the door to re-defining molecular mechanisms of biomolecular interactions in which the membrane bilayer is a key player.

O-195

Antimicrobial peptides: The underlying parameters important for biofilm inhibition and degradation

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Multidrug resistant bacteria to commercial antibiotics are a growing concern worldwide. Such resistance can take place via biofilm formation, sessile micro-colonies formed by many Gram-negative and Gram-positive bacteria, as well as via genetic manipulation that modify the cell wall components. Therefore, it is crucial to investigate new therapeutic strategies and their mechanism of biofilm eradication and killing of bacteria. An attractive alternative to conventional antibiotics is the use of antimicrobial peptides (AMPs), which serve as a component of the innate immune system in all forms of life. Most of these peptides involve in their mode of action the disruption of bacterial membranes, which is believed to overcome bacterial resistance to them. We applied a state of the art multidisciplinary approach combining biochemical, biophysical, and molecular biological studies to shed light into the biophysical properties of AMPs that can; (1) induce genetic changes leading to bacterial resistance to them, and (2) allow their activity against different stages of biofilm life-cycle. We demonstrate the anti-biofilm activity of non-toxic *de-novo* designed AMPs by different mechanisms, including direct killing of sessile bacteria or prevention of bacterial attachment to surfaces.

O-194

Mechanisms of abnormal aggregation and toxicity of amyloid β -protein on neuronal membranes

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The abnormal aggregation of Abeta is considered to cause Alzheimer's disease (AD). Yanagisawa et al. discovered a specific form of Abeta bound to GM1 ganglioside (GAbeta) from the brain of early AD patients, and proposed that GAbeta acts as a template for the formation of Abeta aggregates [1]. This hypothesis was proved in vivo using the anti GAbeta antibody [2]. We have elucidated the following mechanisms for the GAbeta-mediated abnormal aggregation of Abeta by various physicochemical techniques [3]. 1) Abeta specifically recognizes and binds to cholesterol-induced clusters of GM1. 2) At lower Abeta densities on the membrane, the protein assumes an alpha-helix-rich structure whereas at higher densities, it is converted to a beta-sheet-rich oligomer. 3) A further increase in Abeta density leads to the formation of toxic amyloid fibrils, the structure of which is different from that of less toxic fibrils formed in aqueous solution. 4) The less polar environments provided by GM1 clusters play an important role in the formation of these toxic fibrils. In this talk, the details of this process will be discussed.

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O-196 (P-1013)

Single-molecule visualization of dynamic transitions of pore-forming peptides

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Research on the dynamics of single-membrane proteins remains underdeveloped due to the lack of proper approaches that can probe in real time the protein's insertion depth in lipid bilayers. Here we report a single-molecule visualization method to track both vertical insertion and lateral diffusion of membrane proteins in supported lipid bilayers by exploiting the surface-induced fluorescence attenuation (SIFA) of fluorophores. The attenuation follows a d^{-4} dependency, where d is the fluorophore-to-surface distance. The method is validated by observing the antimicrobial peptide LL-37 to transfer among five transmembrane positions: the surface, the upper leaflet, the centre, the lower leaflet and the bottom of the lipid bilayer. These results demonstrate the power of SIFA to study protein-membrane interactions and provide unprecedented in-depth understanding of molecular mechanisms of the insertion and translocation of membrane proteins.

Oral Presentations**– 33. Membrane-active peptides –****O-197 (P-1012)****Beta amyloids aggregation at the surface of model functional membrane domains**

V. Rondelli, E. Del Favero, P. Brocca, L. Cantù
Università degli Studi di Milano, Italy

A hallmark pathological feature of Alzheimer's disease (AD) is the aggregation and deposition of β -Amyloid peptides (A β) in the brain. The interaction of different A β peptides with model membranes with biomimetic composition, mimicking functional domains containing phospholipids, cholesterol and glycolipids, has been studied by different complementary techniques. Calorimetry and X-ray and neutron scattering have been applied to investigate the thermotropic and structural behavior of membranes in bulk, while neutron reflectivity has been applied to study, with the Angstrom sensitivity, the structural interaction of peptides with asymmetric complex membranes prepared by the Langmuir-Blodgett deposition technique. Peptide-membrane interaction was found to depend both on membrane composition and on the state of aggregation of the peptide. Moreover the N-terminal portion of A β was seen to interact with lipids of the bilayer probably promoting the penetration of the peptide in the membrane. Rondelli et al., *Amyloid- β Peptides in interaction with raft-mimic model membranes: a neutron reflectivity insight* Sci. Rep. 6:20997 (2016)

O-198 (P-1011)**Single-molecule microscopy of Staphylococcal pore-forming toxins on live mammalian cells**

A. J. M. Wollman¹, K. Haapasalo^{2,3}, C. de Haas², P. Aerts², E. van't Veld⁴, R. Wubbolts⁴, K. van Kessel², J. van Strijp², M. C. Leake¹

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The majority of Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria produce pore forming toxins, specifically targeting white blood cells (leucocytes), which help infecting cells avoid immune response. Panton-Valentine Leukocidin is one of these toxins and comprises two protein subunits, LukS and LukF, which interact with the human C5a receptor (hC5aR) to form holes in the cell membrane and kill the cell. We have labelled LukS/F and hC5aR with different fluorophores and imaged their interaction in live mammalian cells using rapid single-molecule total internal reflection fluorescence (TIRF) microscopy. We find LukS binds first to hC5aR and forms tetramers within hC5aR clusters. LukF is recruited to these LukS-hC5aR complexes leading to lytic pore formation and simultaneous dissociation of LukS-LukF from hC5aR. Our findings support a crystallographic hetero-octamer model but provide a new view on the kinetics of these crucial virulence factors and their interactions with live cells.

Oral Presentations**– 34. Why disorder matters –****O-199****Mechanistic studies of folding upon binding**

J. Clarke

University of Cambridge, UK

Intrinsically disordered proteins (IDPs) are characterized by a lack of defined structure. Instead, they populate ensembles of rapidly inter-converting conformations with marginal structural stabilities. I will discuss some of our recent studies of folding upon binding. We have discovered that the residual structure content of IDPs is modulated both by ionic strength and by the *type* of ions present in solution. Remarkably, these *ion-specific* structural changes result in binding affinity shifts of up to 6-fold, which happen through alteration of association and dissociation rates. Another feature of IDPs is their promiscuity. Is the mechanism by which they bind a partner protein determined by the sequence of the IDP itself, or by the sequence / structural constraints of the binding partner?

O-200**Investigating the role of N-terminal acetylation on alpha-synuclein structure and function**S. E. Toal¹, A. J. Trexler², D. C. Dewitt², M. A. Brown², E. Rhoades¹¹University of Pennsylvania, Philadelphia, Pennsylvania, USA; ²Yale University, New Haven, Connecticut, USA

Alpha-synuclein (aS) is a small, disordered neuronal protein whose aggregation and deposition as insoluble plaques is one of the primary pathologies associated with Parkinson's disease. Although its native function is still under investigation, thought to involve interactions with cellular membranes. N-terminal acetylation is a ubiquitous post-translational modification of aS (ac-aS), although one that has been neglected in the majority of biophysical studies. Recent NMR studies found that acetylation increases the helical propensity of the N-terminus of aS, but had only minor effects on its interactions with lipid vesicles. Here we seek to better understand the impact of N-terminal acetylation on the physico-chemical properties of aS. To do so, we use NMR, light scattering, and single molecule FRET to contrast aS, ac-aS, and an acetylation mimic, N1-aS. We find that the acetylated protein associates specifically with octyl glucoside and adopts a different topology than unmodified protein upon binding to micelles of this detergent. Our observation provides insight into the role of acetylation in tuning the conformational properties of aS.

O-201**The role of structural disorder in protein degradation *in vitro* and *in vivo***P. Tompa^{1,2}¹VIB-VUB Center for Structural Biology, Brussels, Belgium;²Institute of Enzymology, Budapest, Hungary

Structurally disordered proteins (IDPs) are prevalent in the proteome and often function by partner recognition and induced folding. Structural disorder has a high incidence in chaperones, in which it plays specific mechanistic roles, such as “entropic exclusion” and “entropy transfer” [1]. This is well demonstrated by a specific class of plant “Late embryogenesis abundant (LEA)” proteins involved in stress response [2]. When disordered chaperone fail in mitigating defects of protein folding, structural disorder comes to the rescue in another guise. Regulated protein turnover is regulated by specific signals (degrons), which we suggest to have a “tripartite” nature [3, 4]. Tripartite degrons comprise: (1) a primary degreon that specifies substrate recognition by cognate E3 ubiquitin ligases, (2) secondary site(s) comprising a single, or multiple neighboring, poly-ubiquitinated lysine(s), and (3) a segment that initiates substrate unfolding at the 26S proteasome. We formally demonstrate that all three elements are linked with local structural disorder, which is prevalent even in cases when protein degradation is signaled by unorthodox mono-ubiquitination [5].

[1] Tompa P., Csermely P. (2004) *FASEB J.* 18: 1169-75;[2] Kovacs D. et al. (2008) *Plant Physiol.* 147: 381-90;[3] Guharoy M. et al. (2016) *Nature Comm.* 7: 10239; [4]Guharoy M, Bhowmick P, Tompa P. (2016) *J. Biol. Chem.*291: 6723-31; [5] Braten O. et al. (2016) *Proc. Natl. Acad. Sci.* 113: E4639-47**O-202 (P-1057)****Mapping the link between disorder and function of an IDP-network with single-molecule spectroscopy**

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Intrinsically disordered proteins (IDPs) are ubiquitously found in eukaryotic systems. Their lack of a well-defined structure suggests that their broad conformational ensemble is functionally advantageous. A particularly important system is the IDP-network formed by the basic helix-loop-helix leucine zipper (bHLH-LZ) domains of c-Myc, Max, and Mad, which are major regulators of transcriptome dynamics. Here, we use single-molecule fluorescence resonance energy transfer to investigate the link between the polymer properties of the bHLH-LZ domains of c-Myc, Max, and Mad and the process of coupled binding and folding that leads to functional complexes. In contrast to archetypal IDPs, all three proteins form densely collapsed ensembles under physiological conditions that are dominated by strong attractive electrostatic interactions as quantified using polymer theory. Importantly, the ionic strength sensitivity of the disordered ensembles has pronounced consequences for their functional interactions within the network since salt modulates the binding affinity by almost three orders of magnitude. Our results suggest that the properties of the disordered ensemble and the stability of the functional complexes are strongly correlated.

Oral Presentations

– 34. Why disorder matters –

O-203 (P-1058)**Interplay Between Surface Solvation and Molecular Recognition in IDPs**A. Chowdhury¹, D. Mercadante^{2,3}, P. S. Tan¹, I. V. Aramburu¹, F. Gräter^{2,3}, E. A. Lemke¹¹European Molecular Biology Laboratory, Structural & Computational Biology Unit, Cell Biology & Biophysics Unit, Meyerhofstrasse 1, 69117, Heidelberg, Germany; ²Heidelberg Institute for Theoretical Studies, Schloß-Wolfsbrunnengasse 35, 69118, Heidelberg, Germany; ³Interdisciplinary Center for Scientific Computing, Im Neuenheimer Feld 368, 69120, Heidelberg, Germany

Solvation dynamics tunes bio-molecular dynamics. Such dynamics play a pronounced role for Intrinsically Disordered Proteins (IDPs) as their molecular-recognition goes beyond classical ‘structure-function’ paradigm. We interrogate the interplay between solvation dynamics and molecular-recognition in IDPs; using a combination of site-selective ultrafast fluorescence and all-atom MD simulations. We probed surface water dynamics in IDPs and its attenuation upon partner binding for two IDPs involved in nucleocytoplasmic transport, Nup153FG (N153) and IBB which have as a common binding partner the nuclear transport receptor (NTR) Importin β (I β), despite having different binding modes. N153 binds I β through a set of ultrafast transient multivalent interactions retaining its disorder while IBB forms a helix upon binding I β . Solvent dynamics in N153-I β complex were unperturbed relative to the unbound state while in IBB-I β complex relative slowdown of solvation was seen. This shows a direct correlation between interfacial water dynamics and plasticity of the complexes.

O-204 (P-1059)**Structural and dynamic aspects of antibody recognition of intrinsically disordered antigens**R. S. Norton¹, C. A. Macrauld¹, B. Krishnarajuna¹, R. A. Morales¹, J. Seow¹, N. Drinkwater¹, J. S. Richards², R. F. Anders³, S. McGowan¹¹Monash University, Australia; ²Burnet Institute, Australia;³La Trobe University, Australia

Intrinsically disordered proteins are abundant in *Plasmodium* and related pathogens^[1] and are bona fide vaccine candidates.^[2] Merozoite surface protein 2 (MSP2) is an intrinsically disordered membrane antigen of *P. falciparum*^[3] that elicits a protective response in humans. Crystallography and NMR show^[4] that recognition of a conserved N-terminal epitope by mAb 6D8 is incompatible with its membrane-bound conformation,^[5] suggesting a mechanism by which parasite MSP2 escapes 6D8 recognition. NMR also identifies transient, strain-specific interactions between the 6D8 mAb and more remote regions of MSP2. The conserved C-terminal region of MSP2 is recognised by mAbs 4D11 and 9H4. 4D11 binds to merozoites much more strongly than 9H4. A crystal structure of 4D11 Fv bound to the epitope NKENCGAA reveals the possible conformation of the C-terminal region of MSP2 on the parasite,^[6] underpinning optimization of MSP2 as a vaccine candidate.

[1] Feng et al *Mol. Biochem. Parasitol.* **2006**, 150, 256 [2] MacRaild et al *Structure* **2016**, 24, 148 [3] Zhang et al *J. Mol. Biol.* **2008**, 379, 105 [4] Morales et al *Sci Rep* **2015**, 5, 10103 [5] MacRaild et al *Biochim. Biophys. Acta* **2012**, 1818, 2572 [6] Seow et al *J. Mol. Biol.* **2017**, 429, 836.

Oral Presentations**– 35. Physics of cancer –****O-205****Cancer cell populations in a complex ecology at the timberline of existence**K.-C. Lin³, G. Torga¹, A. Wu², J. Rabinowitz³, K. Pienta³, J. Sturm³, R. Austin³¹Johns Hopkins Medical Institute, Princeton, New Jersey, USA; ²National Institutes of Health, Bethesda, Maryland, USA; ³Princeton University, New Jersey, USA

We have studied interactions and growth in high-drug stress gradients of epithelial and mesenchymal prostate cancer cells. The epithelial-mesenchymal transition (EMT) of cancer cells is thought to play a significant role in invasion and metastasis and is associated with resistance to chemotherapy. Utilizing our engineered microenvironment we sought to simulate a tumor tissue with both epithelial and mesenchymal phenotypes experiencing a chemotherapy stress gradient. It has become clear in our preliminary experiments that cells that survive at the “timberline of existence” at high drug levels are very different from cells in the low drug regions, in a fundamental way: they are multinucleate, and we are able to observe that this multi-nucleation results in the formation of heterokaryons as well. Events including cell division, cell fusion, cell migration, changes in cell morphology and cell motility can be studied at cellular level in real time, and quantitative analyses of these parameters can be made with respect to different spatial distributions or cell populations, thereby providing greater perspective to approach the dynamics in a complex ecology with a heterogeneous fitness landscape

O-207**Cancer research: paradigm instability and the need for a new approach**

J. M. Holly

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There are few people who are unaffected by cancer at some stage in their life. As we live longer the prevalence of clinical cancers continues to rise and many aspects of our ‘Western’ lifestyle accentuate this growth. Over the last 50 years there have been considerable advances in our understanding, but limited progress has been made in tackling the clinical disease. Over this period the main focus of research has been on understanding the molecular genetics of cancer. Advances in technology have facilitated the discovery of considerable detail regarding the nature of cancer at the molecular and cellular level. This understanding has resulted in many new targeted-therapies, but their success is generally measured in prolonging survival by just a few months. Recent discoveries have provided some explanations for this limited progress but they have also challenged many of the dogmas that have underpinned the research of the last decades; indicating the need for new approaches. New questions have arisen, with challenges of complexity and heterogeneity, that require new thinking. Emerging evidence indicates that the development of clinical cancers is more affected by our lifestyles than by our genes: raising the prospect that prevention may be more successful than treatment.

O-206**Cancer treatment as perturbation of a complex dynamic system: exploiting evolutionary dynamics to optimize therapy**

R. A. Gatenby

Moffitt Cancer Center, Tampa, Florida, USA

A number of successful systemic therapies are available for treatment of disseminated cancers. However, tumor response to these treatments is almost invariably transient and therapy fails due to emergence of resistant populations. The latter reflects the temporal and spatial heterogeneity of the tumor microenvironment as well as the evolutionary capacity of cancer phenotypes to adapt to therapeutic perturbations. Interestingly, although cancers are highly dynamic systems, cancer therapy is typically administered according to a fixed, linear protocol. Treatment is changed only when the tumor progresses but successful tumor adaptation begins immediately upon administration of the first dose. Applying evolutionary models to cancer therapy demonstrate the potential advantage of using more dynamic, strategic approaches that focus not just on the initial cytotoxic effects of treatment but also on the evolved mechanisms of cancer cell resistance and the associated phenotypic costs. The goal of evolutionary therapy is to prevent or exploit emergence of adaptive tumor strategies. Examples of this approach include adaptive therapy and double bind therapy. The former continuously alters therapy to maintain a stable tumor volume using a persistent population of therapy-sensitive cells to suppress proliferation of resistant phenotypes. The latter uses the cytotoxic effects of an initial therapy to promote phenotypic adaptations that are then exploited using follow-on treatment. In pre-clinical models, application of adaptive therapy permits indefinite tumor control with a single cytotoxic drug. Clinical results from studies using adaptive therapy and double bind therapy will be presented.

O-208 (P-1074)**The application of scanning near field infrared microscopy to cancer**

P. Weightman

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There is an international need to improve the diagnosis of cancer. Traditional methods rely on the examination of excised tissue by histologists, two of whom, for some cancers, typically agree on a diagnosis only 70% of the time. False positives can lead to serious but unnecessary surgery and false negatives can be fatal. Fortunately it is becoming clear that the diagnosis of cancer can be improved by analysis techniques based on the examination of tissue using infrared (IR) imaging technology. Recently it has been shown that two methods of IR imaging, scanning near field optical microscopy (SNOM) using an IR free electron laser [1] and Fourier transform IR (FTIR), are able to discriminate between normal, dysplastic and cancerous cervical tissue [2]. The two IR imaging approaches have different strengths and weaknesses. FTIR yields information from several thousand wavelengths but at poorer spatial resolution whereas SNOM provides excellent spatial resolution but over a smaller number of wavelengths. The two techniques will be compared in studies of a single cancer cell.

1 A.D. Smith, et. al. *App. Phys. Lett.* **102** 053701 (2013)2 D.E. Halliwell, et.al. *Nature Scientific Reports*, **6** 29494 (2016)

Oral Presentations**– 35. Physics of cancer –****O-209 (P-1075)****Cancer risk and the tree of somatic cell divisions**
I. Derenyi¹, G. J. Szollosi^{1,2}¹Department of Biological Physics, Eotvos University, Budapest, Hungary; ²MTA-ELTE “Lendulet” Evolutionary Genomics Research Group, Eotvos University, Budapest, Hungary

All cells of an individual are the result of cell divisions organized into a single binary tree. This somatic cell tree is not uniform: some branches are shorter, while others can become much longer. As cell divisions are accompanied by replication errors, longer cell lineages are more prone to the accumulation of mutations and to carcinogenesis. By mapping the accumulation of driver mutations along a somatic cell tree into a graph theoretical problem, we derive a mathematical expression for the probability of developing cancer in an arbitrary cell tree with a given mutation rate. The result is consistent with epidemiological data and highlights the significance of the longest cell lineages. We also show how tissues can minimize the length of their longest lineages through differentiation hierarchies.

O-210 (P-1076)**AFM and graph analysis to study P-cadherin/SFK mechanotransduction signalling in breast cancer cells**F. A. Carvalho¹, A. S. Ribeiro², J. Figueiredo², R. Carvalho², T. Mestre³, J. Monteiro², A. F. Guedes¹, M. Fonseca³, J. Sanches³, R. Seruca², J. Paredes², N. C. Santos¹¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal; ²i3S / IPATIMUP, Universidade do Porto, Portugal; ³IST-ISR, Universidade de Lisboa, Portugal

Forces mediated by cell-cell adhesion molecules, as cadherins, play a crucial role in preserving normal tissue architecture. In breast cancer, P-cadherin overexpression increases *in vivo* tumorigenic ability, as well as *in vitro* cell invasion, by activating Src family kinase (SFK) signalling. It is not known how P-cadherin and SFK activation impact cell-cell biomechanical properties. Using atomic force microscopy (AFM) images, cell stiffness and cell-cell adhesion measurements, and undirected graph analysis, we demonstrate that P-cadherin overexpression promotes significant alterations in cell's morphology, by decreasing cellular height and increasing its area. It also affects biomechanical properties, by decreasing cell adhesion and stiffness. Cell network analysis showed alterations in intercellular organization, which is associated with cell adhesion dysfunction, destabilization of an E-cadherin/p120ctn membrane complex and increased cell invasion. Remarkably, inhibition of SFK signalling, using dasatinib, reverted these pathogenic P-cadherin induced effects. [Ribeiro *et al.* (2016) *Nanoscale* **8**, 19390-401]

Posters

– 1. Multiscale biophysics of membranes –

P-1 (O-13)

Developing ESCRT-III as a toolkit for bottom-up construction of eukaryote-like artificial cells

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The Endosomal Complex Required for Transport (ESCRT) is a ubiquitous class of proteins involved in most membrane remodeling processes in eukaryotic cells. Previous studies have demonstrated that ESCRTs can be reconstituted in artificial lipid vesicle systems, notably giant unilamellar vesicles (GUVs), where they are able to induce the formation of intraluminal vesicles (ILVs), encapsulating material from the extravesicular solution.^[1]

Our aim is to develop the ESCRT proteins as a toolkit to generate GUV-derived structures, with multiple compartments containing different chemical environments, enzymes or molecular probes. The resulting cell-like structures may find applications in synthetic biology, drug delivery, diagnostics and the development of nano-reactor technology.^[2]

Using confocal microscopy and flow cytometry, we have gained insights into how protein stoichiometry and membrane mechanics influence the size and number of the ILV compartments obtained. We will further develop the ESCRT toolkit using the ATP-ase Vps4 to induce multiple encapsulation events and introduce rationally designed ESCRT chimera proteins to further simplify the system.

[1] T. Wollert, J. Lippincroft-Schwartz, J. H. Hurley, *Nature*, 2009

[2] P. A. Beales, B. Ciani, *Phys. Chem. Chem. Phys.*, 2015

P-3 (O-15)

Cholesterol and polyunsaturated lipids working in concert to modulate G protein-coupled receptors

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G protein-coupled receptors (GPCRs) are signaling machines that constitute the largest family of integral membrane proteins in human genome. Given that many GPCRs contain binding sites for specific lipids, there is reason to assume that lipids play a key role in the stability, dynamics, and activation of various GPCRs. However, the critical barrier to progress in the field has been the lack of data showing in atomistic detail how *exactly* lipids modulate GPCRs. Here we overcome this challenge through extensive self-assembly and equilibrium simulations of both atomistic and coarse-grained models of multi-component lipid membranes with GPCRs. Through considerations of many proteins (e.g., the human β_2 -adrenergic receptor, the adenosine A2A receptor, etc.), we discuss how GPCRs reorganize their membrane environment to create domains that promote their function. In these domains, cholesterol is one of the central lipids, and we discuss how it can modulate GPCR stability, dynamics, and conformation in an allosteric fashion. Meanwhile, lipids with polyunsaturated fatty acids turn out to be decisive in modulating GPCR partitioning in different membrane environments. Overall, lipids modulate GPCRs in a largely concerted manner that becomes evident in multi-component membrane regions.

P-2 (O-14)

Anomalous diffusion in artificial lipid bilayers

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In contrast to artificial bilayers, diffusion in cells is slower and non-ergodic. The physical origins of anomalous diffusion are poorly understood; cytoskeletal-membrane interactions and nanoscopic roughness have been suggested as mechanisms. We use a bottom-up approach to create mimics of anomalous behaviour to better understand the factors that control diffusion. Using a combination of single-molecule fluorescence and interferometric scattering microscopy we study diffusive behaviours at microsecond time resolution over several seconds.

In a supported bilayer model with PEG-modified lipids, we can control free diffusion: as the concentration of PEG-lipids increases, bilayer contiguity is disrupted. We observe a dramatic and controllable switch in anomalous diffusion as we cross the percolation threshold that is dependent on the length of PEG modification. Our results are well described using a fractal model of tracer diffusion.

To address the role of topography, we generate bilayers on glass substrates with square-wave 'nanowall' patterning. Although fluorescence tracking reveals anisotropic diffusion, this is not anomalous over any timescale. This reveals that topological confinement, as opposed to topography, is required for anomalous diffusion in lipid bilayers.

P-4

Interaction between a synthetic antitumoral catechin and anionic phospholipids membranes

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A synthetic derivative of a green tea polyphenol, 3-O-(3,4,5-trimethoxybenzoyl)-(-)-catechin-3-gallate (TMCG) has showed antiproliferative activity against malignant melanoma. The hydrophobic nature of TMCG suggests that the interaction with membranes would be important to understand the molecular mechanism, and its potential capacity to modulate membrane related processes. We study the interactions of TMCG with model anionic phospholipids membranes (dimiristoylphosphatidylserine and dimiristoylphosphatidylglycerol) by using different biophysical techniques. Differential scanning calorimetry showed that TMCG perturbs the gel to liquid crystalline phase transition of the phospholipids. Infrared experiments showed that TMCG has no marked effect on the fluidity of the methylene chains of the phospholipids but it produces an important increase in the hydration of the interfacial region of the bilayer in the fluid phase. Fluorescence polarization showed that TMCG increases the order in the upper part of the bilayer in the fluid phase. These results support the idea that TMCG incorporates into anionic phospholipid bilayers and produces structural perturbations which might affect the function of the membrane.

Acknowledgement

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Posters**– 1. Multiscale biophysics of membranes –****P-5****Solid state NMR investigations and MD simulations of triblock copolymers in lipid bilayers**R. Baerenwald¹, T. M. Ferreira¹, S. O. Ollila^{2,3}, K. Saalwächter¹¹Institute of Physics – NMR, Martin-Luther-University Halle-Wittenberg, 06120 Halle, Germany; ²Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 16610 Prague, Czech Republic; ³Institute of Biotechnology, University of Helsinki, 00014 Helsinki, Finland

Pluronics are triblock copolymers widely used in pharmaceutical industry that consist of hydrophilic poly(ethylene oxide) end blocks and a hydrophobic poly(propylene oxide) middle block. Using solid-state nuclear magnetic resonance (NMR), we investigated the structure and dynamics of different Pluronics interacting with a number of phosphatidylcholine membranes with variable bilayer thickness and acyl chain saturation. ¹H-¹H Nuclear Overhauser Enhancement (2D-NOESY) experiments were applied to confirm the insertion of the copolymer into the bilayer. Information on the dynamically averaged structure of lipid and polymer segments was gained from measuring motionally averaged dipolar couplings. Specifically, we used the Back-to-Back (BaBa) double-quantum recoupling pulse sequence [1] to determine the very small homonuclear ¹H-¹H residual couplings of the polymers. In this way, we were also able to quantify the relative amount of inserted vs non-inserted polymer. By using different molecular dynamics (MD) simulation models to determine the NMR dipolar couplings and relaxation rates measured, we get very detailed information about how the polymer molecules incorporate in the bilayer.

[1] K. Saalwächter et al., J. Magn. Reson., 212, 204, 2011

P-7**Bending elasticity of lipid membranes at extremely high curvatures**P. Bashkirov^{1,2}, A. Shnyrova³, K. Chekashkina^{1,2}, P. Kuzmin², V. Frolov^{3,4}¹Federal Research and Clinical Center of Physical-Chemical Medicine, Russian Federation; ²A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of RAS, Russian Federation; ³Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, Spain; ⁴IKERBASQUE, Basque Foundation for Science, Spain

Highly curved membrane structures are constantly formed during membrane remodeling in cells. As these curved intermediates have inherently limited size, confined to almost molecular dimensions, their transformations elude direct experimental observation. We show here that such nanoscale membrane processes can be emulated and quantified using electro-elastic coupling in membrane nanotubes (NTs). The electric field driving ionic current through the nanotube lumen changes the NT curvature, the resulting conductance non-linearity being a measure of elasticity for nanoscopic-scale deformations. Lipid bilayer deformations can be described using linear elastic approximation up to extreme curvatures where NT spontaneous collapse occurs. Comparing the field effects at low and high NT curvatures we concluded that macroscopic elasticity correctly describes the energetics of nanoscopic membrane deformations with extreme curvature values. This work was supported by RSF grant 14-14-01001.

P-6**Model membrane systems for protein therapeutics**H. M. Barriga¹, O. Ces², R. V. Law², N. J. Brooks², J. M. Seddon², M. M. Stevens^{3,1}¹Division of Biomaterials and Regenerative Medicine, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden; ²Department of Chemistry, Imperial College London, London, UK; ³Departments of Materials and Bioengineering and Institute of Biomedical Engineering, Imperial College London, London, UK

Cellular membranes are formed from complex and highly regulated mixtures of lipids and proteins. Lipids exhibit rich phase morphology which affects their association and binding to proteins and can impact cellular dynamics and signaling pathways. Model membrane systems can be used to mimic and understand this behavior to characterize lipid polymorphism such as bicontinuous cubic and hexagonal phases. Dispersions of these phases in the presence of a stabilizing polymer form highly stable nanoparticles with high membrane surface area to volume ratios and a significantly higher number of lipid molecules compared to vesicular systems. This enables their application for the delivery of protein therapeutics and lipid signaling molecules. We focus on the structural effects of loading lipid nanoparticle dispersions with large bioactive molecules and their interactions with CCL1 cell lines. Structural changes and loading of the nanoparticles have been characterised using a combination of Small Angle X-ray Scattering, Dynamic Light Scattering and FRET experiments. Cellular interactions have been characterized using fluorescence spectroscopy and microscopy measurements to investigate cytotoxicity and uptake.

P-8**A microfluidic platform to study model biomembranes**

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The development of model cellular membranes is crucial for fundamental investigations of biological phenomena, ranging from antimicrobial peptide pore formation and biomolecule transport to bilayer elasticity and lipid raft formation. In this presentation, I will discuss my recent work establishing a new approach to study phospholipid bilayers in a platform where the membrane properties can be controlled and interrogated in a rational manner. The method relies on an adapted thin-film balance apparatus allowing for the formation of a free standing black lipid membrane in the center of an orifice surrounded by microfluidic channels. A distinguishing feature of this technology is the robust creation of tension-controlled membranes with millimeter-scale surface area in a planar geometry that can be simultaneously imaged and manipulated, for example by introducing membrane-active molecules. Case studies highlighting the advantages and versatility of the approach will be presented, including measurements of bilayer elasticity, phospholipid phase separation, and membrane-active peptide pore formation. Together, the results demonstrate a new paradigm for studying the mechanics, structure, and function of biological membranes.

Posters

– 1. Multiscale biophysics of membranes –

P-9

Solubilization of trans-membrane proteins by styrene-maleic acid (SMA) copolymersA. Bérczi¹, R. Domokos¹, Á. Deák², Z. Szegletes¹, L. Janovák², I. Dékány², L. Zimányi¹¹Institute of Biophysics, BRC, Hung. Acad. of Sci., Szeged, Hungary; ²Dept. of Phys. Chem. & Materials Sci., Univ. of Szeged, Szeged, Hungary

There are many agents capable of solubilizing *trans*-membrane proteins. However, integral membrane proteins can function only when they are surrounded by special lipids. Recently it has been shown that some styrene-maleic acid copolymers [i.e. SMA(2:1) and SMA(3:1)] are polyelectrolyte amphiphils that can create microdomains in which integral membrane proteins are kept together with surrounding lipids. We were interested in whether SMA copolymers are capable of solubilizing integral membrane proteins and creating microdomain structure. We have been long interested in producing recombinant *trans*-membrane proteins (cytochromes *b561*) in yeast cells and solubilizing them by nonionic detergents. In this work we compare the parameters of solubilized particles obtained by using SMA(2:1), SMA(3:1) copolymers and dodecyl-maltoside (DDM) non-ionic detergent. The results obtained by UV/Vis spectroscopy, dynamic light scattering technique, atomic force and transmission microscopy allow us to show that (1) solubilization efficacy by SMA(2:1) is comparable with that by DDM, (2) SMA copolymers highly affect the interaction of the proteins in microdomains with negatively charged substrates, and (3) the size of particles obtained by different solubilizing agents are very inhomogeneous.

P-11

Order of monogalactolipid α -linolenoyl acyl chains in the lamellar and inverted hexagonal phasesL. Bratek¹, J. Jalocho-Bratek², K. Baczynski², M. Pasenkiewicz-Gierula²¹Institute of Nuclear Physics, Polish Academy of Sciences, Poland; ²Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Poland

Photosynthesis is one of the most important processes on Earth. It takes place in thylakoids – organelles that are surrounded by photosynthetic membranes consisting in ~75% of galactolipids. The most abundant galactolipid of the membrane is monogalactosyldiacyl-glycerol (MGDG) with α -linolenoyl (di-18:3, *cis*) acyl chains. MGDG, due to its conical shape, promotes formation of an inverted hexagonal phase (H_{II}).

In this molecular dynamics (MD) simulation research, two computer models were constructed; one of the inverted hexagonal phase of MGDG and the other of a lamellar MGDG bilayer. Each of the models contained ~800 MGDG molecules and 30 water molecules per MGDG and was MD simulated for nearly one μ s. The equilibration of the H_{II} system required ~400 ns of MD simulation. The 500-ns production trajectory of each system was analysed to obtain molecular and deuterium order parameters, cross-sectional area per lipid and other basic structural parameters. The parameters for each phase are compared and their differences are explained based on the spatial properties of each phase.

P-10

Axon-glia interactions and their role in regulation of conduction of impulses of nerve fibers

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Myelinated nerve fibers were studied using Raman's spectroscopy. It is well known that changes in Raman spectrum would show changes in viscosity of myelin. In addition, Schwann cells have protein S-100, which acts similar to calmodulin, changing the amount of calcium in myelin and maintaining ordered structure of myelin. In order to get into Schwann cell's myelin layers and to add antibodies to protein S-100 for specific binding, it would be necessary to make Schwann cell to burst with osmotic shock. Experiments were conducted and the results have been shown, where incubation of nerve fibers in hypoosmotic solution with low antibody content to protein S-100 towards control solution, imitating physiological circumstances (Ringer's solution). Condition of myelin changes is unauthentic. Addition of high concentrated dose of antibodies showed that myelin sheet structure changes even more and changes are authentic.

P-12

Statistics of individual leakage events to consistently interpret vesicle leakageS. Braun², Š. Pokorná³, R. Sachl³, M. Hof³, H. Heerklotz^{1,2}, M. Hoernke^{1,2}¹BIOSS Centre for Biological Signalling Studies, University of Freiburg, 79104 Freiburg i. Br., Germany; ²Department of Pharmaceutical Technology and Biopharmacy, University of Freiburg, 79104 Freiburg i. Br., Germany; ³J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, 182 23 Prague, Czech Republic

Many antimicrobial compounds or drugs act by permeabilizing the lipid portion of membranes. Typically, membrane permeabilization is modelled by the release of an entrapped dye from lipid vesicles. Two recent assays are sensitive to the distribution of leakage over the vesicle population: (1) The microscopic quantification of dye influx into giant unilamellar vesicles (GUVs) and (2) the fluorescence lifetime-based leakage of large unilamellar vesicles (LUVs). The two experimental approaches differ systematically in the observed extent and mechanism of leakage. We resolve these apparent discrepancies and better exploit the potential of both methods by considering leakage as a result of individual leakage events (LEs) of a certain abundance and intrinsic permeability also taking into account the vesicle size. Importantly, we establish a concept valid for leakage events continuously ranging from small and short-lived membrane defects (graded leakage) to very large and stable pores (all-or-none type leakage). A general concept for leakage permits greatly improved conclusions regarding biological activity of membrane-active molecules.

Posters

– 1. Multiscale biophysics of membranes –

P-13

Influence of lipid saturation and head group charge on the binding of a short antimicrobial peptideD. Ciumac¹, R. A. Campbell², L. A. Clifton³, H. Xu⁴, J. R. Webster³, J. R. Lu¹¹School of Physics and Astronomy, Univ. of Manchester, UK; ²Institut Laue Langevin, Grenoble, France; ³ISIS Neutron Facility, STFC, Chilton, Didcot, UK; ⁴Centre for Bioengineering and Biotechnology, China Univ. of Petroleum, Qingdao, China

Many antibacterial peptides (AMPs) target bacterial membranes and kill them by causing structural disruptions [1]. Key differences between the bacterial and mammalian membranes are the charge characteristics and the lipid acyl chain length and saturation, as bacteria are modifying their membranes to adapt to environment changes [2]. In this study we report the results concerning the binding of a short designed AMP, with the general sequence G(IKK)4I-NH₂ to spread lipid monolayers of saturated zwitterionic DPPC, and anionic DPPG and unsaturated anionic POPG mimicking the charge difference and acyl chains saturation between membranes. The peptide binding properties vary considerably with head group charge and tail saturation. The peptide penetrated the anionic lipid monolayers and removed lipids from the interface, associating with both head group and tail regions. However, in case of DPPC, the peptide was only associated with the head groups and the lipid removal was negligible. The results are discussed in the context of general antibacterial actions and membrane lytic processes [3].

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- [2] A. Mrozik et al, *Pol. J. Environ. Stud.*, 13, 487–494, 2004.
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P-15

Transbilayer coupling in gel-fluid asymmetric lipid vesiclesB. Eicher^{1,2}, I. Letofsky-Papst^{3,4}, D. Marquardt^{1,2}, E. London⁶, J. Katsaras⁵, G. Pabst^{1,2}

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We applied small- and wide-angle x-ray scattering (SWAXS), small-angle neutron scattering (SANS), differential scanning calorimetry (DSC) and cryo-electron microscopy (cryo-EM) to study asymmetric large unilamellar vesicles (aLUVs) composed of palmitoyl oleoyl phosphatidylethanolamine (POPE)/ palmitoyl oleoyl phosphatidylcholine (POPC). Independent melting of both leaflets was observed. However, compared to the thermotropic behavior of the single lipid components and their symmetric mixtures, transition peaks were significantly shifted in aLUVs, indicating transbilayer coupling. In particular, we found that a coexisting fluid phase leads to a decrease of the lipid packing density in the gel phase. However, the effect is not strong enough to fully break-up the hydrogen bond-stabilizing network formed by PE headgroups.

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P-14

Lipid nanotubes pulled and pushed from free-standing lipid membranesA. Dols-Perez, V. Marin, R. Kieffer, M.-E. Aubin-Tam
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Lipid nanotubes play a vital structural role in different cellular organelles such as the endoplasmic reticulum, mitochondria and Golgi apparatus, but also in communication processes such as inter and intracellular exchanges and cellular migration. The study of their biophysics is often carried on vesicles, supported lipid bilayers or living cells. In these approaches, it is challenging to achieve asymmetric lipid distribution, dynamic buffer control and zero curvature. In contrast, the use of a freestanding lipid bilayer on a chip present additional advantages such as easy access to both sides of the membrane, possibility to create several membranes in a same device, possibility to circulate different solutions, and full compatibility with optical techniques.

We show a novel method to study lipid nanotubes by combining optical tweezers with freestanding lipid membranes formed inside a microfluidic chip. The bilayers were assembled over a hole inside a microfluidic device and lipid nanotubes were formed via two methods: 1) by pulling a lipid patch anchored to bead by streptavidin-biotin interactions or 2) by pushing the bead across the membrane. The tension, bending rigidity and the force required to pull the nanotubes will be discussed.

P-16

New insights into cholesterol's influence on lateral segregation in bilayers

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Lateral segregation into ordered and disordered domains may occur when mixing unsaturated and saturated phospholipids (PLs), especially in the presence of cholesterol. Generally, cholesterol prefers sphingomyelin (SM) over saturated phosphatidylcholines (PC), saturated over mono- or polyunsaturated PLs. Our aim was to understand how cholesterol's affinity for different PLs influence lateral segregation. Fluorescent sterol partitioning experiments were used to determine cholesterol's relative affinity between unsaturated and saturated PLs. Using time-resolved fluorescence of trans-parinaric acid we determined the amount of saturated PLs required to form ordered domains in fluid bilayers containing 0 or 20 mol% cholesterol to compare to what degree cholesterol promoted lateral segregation. For unsaturated PLs, we varied the headgroup and the degree of unsaturation. For saturated PLs, di-16:0 PC, SMs with different *N*-acyl chain lengths and biological SM mixtures were compared. In conclusion, the degree to which cholesterol promoted domain formation was dependent on cholesterol's relative affinity between the unsaturated and saturated PLs for a vast variety of tested PLs. Biological impact and general observations about cholesterol influence on domain formation will be presented.

Posters

– 1. Multiscale biophysics of membranes –

P-17

The notion of catalysis in membrane remodellingV. Frolov^{1,2}, A. Shnyrova¹, P. Bashkirov^{3,4}

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Fusion and fission of cellular membranes involve extreme deformations of the lipid matrix. Creation of focused curvature stress is required to trigger formation of generic non-bilayer intermediates of disparate fusion and fission reactions in cells. While localized membrane bending is generally a consequence of a mechanical or mechano-chemical activity of specialized proteins, actual topological transition involves a catalytic element related to differential stabilization of non-bilayer membrane intermediates by the protein machinery. In order to discriminate mechanical and catalytic reaction steps we analyzed membrane fission mediated by different proteins and by pure mechanical forces. We determined specific features of the membrane fission catalysts and discuss emergence of them in the evolutionary development of the protein fission machinery.

P-19

Catch me if you can: microfluidic traps for studying biomolecular processes in GUVsK. A. Ganzinger¹, M. Heymann¹, T. Robinson², P. Schwille¹

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Cell-sized giant unilamellar vesicles (GUVs) from natural or non-natural lipids are widely used as a model system for cellular membranes. With the goal of an ever-better understanding of processes at biomembranes, researchers reconstitute increasingly complex biomimetic systems in GUVs. However, it is still not trivial to generate GUVs with an asymmetrically functionalised membrane, often a basic requirement for biological mimicry. In particular, electroformation creates symmetric GUVs by default, requiring subsequent introduction of asymmetry which is hampered by their fragility. Here, we present a microfluidic chip for entrapment of GUVs in 1mm-wide channels using PDMS posts. Our design of post positioning maximises trapping efficiency, so even for GUV preparations with low yields high-quality GUVs can be readily observed. On-chip capture of GUVs allows to control the composition of the external solution, thereby enabling detachment of proteins specifically from the outer GUV membrane leaflet. Unspecific GUV and protein adhesion to the traps was prevented by PDMS coating. Our improved microfluidic traps provide an efficient tool for GUV manipulation during microscopic observation, increasing throughput and opening new avenues for experimental studies of biomolecules in GUVs.

P-18

Understanding the lyotropic phase behaviour of cytochrome-c incorporated in monoolein mesophasesA. Gainar¹, A. I. I. Tyler², N. J. Brooks¹, J. M. Seddon¹

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The hydrated monoolein structures have been previously investigated for a long period of time to confirm their bicontinuous cubic phases that are, in fact, matrices able to entrap, protect, deliver and slowly release various molecules with biological importance. As drug efficacy depends on the mechanism of incorporation of such biomolecules into cubic self-assemblies, it is necessary to understand the processes of phase transformation as much as the lipid-protein interactions. This study presents the phase changes of a monoolein-incorporated small protein, cytochrome-*c*, which is commonly associated with the inner mitochondrial membrane and is an important component of the respiratory electron transport chain. To obtain the systems of interest, mixtures of various wt % ratios were prepared using monoolein, cytochrome-*c* and a Tris-HCl buffer (pH 7). By keeping the cytochrome-*c* at constant composition of 5%, but varying the monoolein composition, as well as the temperature and pressure, changes in the SAXS diffraction patterns are noticeable.

P-20

The vertical location of tocopherol is not altered as a function of membrane lipid unsaturation

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We have studied the location of alpha-tocopherol in model membranes formed by different unsaturated phosphatidylcholines, namely POPC, PLPC, PAPC and PDPC. In all the cases only one lamellar phase was detected by X-ray diffraction. Results from quenching of α -tocopherol intrinsic fluorescence by acrylamide showed that this vitamin was inefficiently quenched in the four types of membranes, indicating that the fluorescent chromanol ring was poorly accessible for this hydrophilic quencher. Compatible with that, quenching by doxyl derivatives of phosphatidylcholines indicated that the chromanol ring was close in the four membranes to the nitroxide probe located at position 5. ¹H-MAS-NMR showed that α -tocopherol induced chemical shifts on protons from the phospholipids, especially of those bonded to carbons 2 and 3 of the acyl chains of the four phospholipids studied. ¹H-MAS-NMR NOESY results suggested that the lower part of the chromanol ring was located between C3 of the fatty acyl chains and the centre of the hydrophobic monolayer for the four phospholipid membranes studied. Taken together, these results suggest that α -tocopherol is located, in all the membranes studied, with the chromanol ring within the hydrophobic palisade but not far away from the lipid-water interface.

Posters

– 1. Multiscale biophysics of membranes –

P-21

Why do lipid domains register?

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Model systems such as supported lipid bilayers have been instrumental in understanding the fundamentals of how lipids organise themselves. Phase separated micro-domains typically form symmetrically, aligning precisely across the leaflets. This is known as domain registration. However, there is a poor understanding of inter-leaflet coupling mechanisms, with little direct experimental data. Recent simulations have shown that increasing hydrophobic mismatch should increase the tendency of lipid domains to form the asymmetric anti-registered state, in order to minimise the increasing line tension.

Our aim is to drive the system to spontaneously form in an anti-registered state, and therefore understand the magnitude of the forces driving registration. We increase line tension by systematically increasing the chain length of the saturated lipid in a ternary lipid mixture. AFM is used to image supported lipid bilayers, distinguishing different lipid domains by their height and mechanical response. This approach has proven successful, and we present images of anti-registered supported lipid bilayers. AFM force spectroscopy is used to distinguish between the two leaflets of a bilayer, through which the orientation of the anti-registered region can be determined.

P-23

Gold nanowire fabrication with lipid nanotubes

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The fabrication of conductive nanostructures is the key technology in semiconductor industry and has gained importance in biology for applications such as biosensors and drug delivery.

We have demonstrated a high-throughput approach to fabricate gold nanowires on surfaces with a lipid nanotube template¹. Biotin-tagged lipid nanotubes are formed from lipid blocks in inverted hexagonal phase adsorbed on polymer-coated surfaces upon application of shear force. Streptavidin-coated gold nanoparticles were attached to the biotin-tagged lipid nanotubes and gold nanoparticle-encapsulated lipid nanotubes were cross-linked by chemical fixation. Samples were dried and treated with oxygen plasma to remove the organic template and connect the particles. The created nanowires were characterized by cryo-transmission electron microscopy, atomic force microscopy and electrical measurements. The approach can further be combined with single lipid nanotube patterning with a micromanipulator² to create distinct patterns instead of random networks.

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P-22

Caveolae dynamics is strongly influenced by the lipid composition of the plasma membrane

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Caveolae are flask-shaped structures of the plasma membrane (PM), which are rich in cholesterol and sphingolipids. They are abundant in fat cells and have been linked to lipid metabolism. Biogenesis of caveolae is mainly driven by the membrane protein caveolin1 and the cavin-coat complex while their dynamics is controlled by the ATPase EHD2. Despite considerable research, little is known about effects of certain lipids on caveolae dynamics. Here we aim to understand how the proteins cooperate with lipids to regulate the equilibrium between stable and dynamic caveolae. By using fusogenic liposomes we can directly insert fluorescently labeled lipids into the PM and follow the distribution using microscopy. A stable mammalian cell line expressing caveolin1-mCherry allows monitoring caveolae and their dynamics before and after lipid addition using live cell TIRF microscopy. Results show that sphingomyelin stabilizes caveolae at the cell surface whereas lactosylceramide and GM1 greatly enhance their mobility. Visualisation of caveolae associated endogenous proteins with immunofluorescence revealed a crucial role of EHD2 in coping with excess lipids. The knowledge gained from this work will be applied in studies using adipocytes to help understand the role of caveolae in lipodystrophy.

P-24

Determinants of sodium and calcium adsorption onto neutral lipid bilayers

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Metal cations adsorption to cellular membranes change a number of key functions, such as interaction with charged moieties, cell volume, membrane fusion or cell membrane potential. However, it is unclear how or whether cells regulate this adsorption and hence the related functions through adjusting the local lipid composition of their membranes. We have employed both fluorescence techniques and computer simulations to study how the presence of cholesterol -- a key molecule in inducing membrane heterogeneity -- and temperature can affect the adsorption of sodium and calcium on neutral phosphatidylcholine (PC) bilayers. We find that whereas transient sodium binding is dependent on the sole number of exposed PC head groups, the strong adsorption of calcium is determined by the available surface area of the membrane. Notably, cholesterol plays an indirect role in enlarging the total membrane area, therefore, increasing calcium adsorption, while having no effect on the adsorption of sodium. These findings improve our understanding of how lateral lipid heterogeneity regulates numerous ion-induced processes including adsorption of peripheral molecules such as the usually charged components of the glycocalyx.

Posters

– 1. Multiscale biophysics of membranes –

P-25

Cholesterol-like effects on DPPC bilayers induced by a fluorotelomer alcohol

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Fluorocarbon amphiphiles are anthropogenic molecules present in many applications such as food packaging, clothing and cookware. The non-biodegradability and potential bioaccumulation of these molecules in biological membranes has motivated concerns about their biological effects, however, little is known specially on the molecular level. We present an experimental study based on solid-state nuclear magnetic resonance (NMR) spectroscopy, differential scanning calorimetry (DSC) and confocal laser scanning microscopy (CLSM) to investigate the effect of a fluorocarbon amphiphile (1H,1H,2H,2H-perfluoro-1-decanol) on DPPC bilayers as function of temperature and concentration. The fluorinated alcohol induces a two-phase coexistence below the gel to liquid-crystalline phase melting temperature (T_m) and a strong ordering of the phospholipid acyl chains above melting. The DSC results are very similar to analogous measurements done on DPPC/sterol bilayers and the condensing effect on the phospholipid acyl chains is of similar magnitude as for cholesterol at the same concentration. The set of results presented indicates that the fluorocarbon studied behaves essentially as an amphiphilic rigid rod with similar effects as sterols on phospholipid bilayers.

P-27

Design of a switchable DNA origami structure for shaping lipid membranes

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Biological membranes are dynamic cellular barriers that suffer deformation and bending. In recent years, due to its exclusive nano-engineering properties, the DNA origami technology has been vastly used to build synthetic scaffolds that partially recapitulate curvature-inducing mechanisms. Nonetheless, the control over such shaping phenomena is yet scarce. Here, we design a DNA based nanostructure with an integrated conformational switch. Through site directed single-strand displacement reactions, membrane bound structures can change their conformation into a bent state, with the goal to deform the lipid membrane. By variation of nucleotide sequence different bending angles can be achieved. Furthermore, current theoretical predictions of membrane bending energies can be confirmed through comparison with the free energy changes of the bound DNA structures.

P-26

Time-dependent phase diagram of bolaamphiphile molecules presenting various lamellar structures

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Phase diagram of synthetical bolaamphiphile molecules issued from natural resources and based on N-(12-Betainylamino-dodecane)-octyl- β -D-Glucosyluronamide Chloride is investigated. Fine tuning of chemical structure leads to different macroscopic organizations of these molecules. The length of the main bridging chain and the lateral chain are varied in order to modify the hydrophilic-lipophilic balance. A diacetylenic unit is introduced in the middle of the bridging chain to study the influence of the π - π stacking on the supramolecular organization of these molecules. The phase diagram of bolaamphiphiles is presented as they self-organize in supramolecular structures such as lamellar crystalline structure, L_c , lamellar gel structure, $L_{\beta'}$, lamellar fluid structure, L_{α} , and lamellar isotropic structure, L . Thermal hysteresis of these structures, following phase transitions, is investigated by small-angle and wide angle X-ray scattering. The phase diagram is time-dependent because the system remains in the kinetically stabilized undercooled high-temperature phase at ambient temperature for many hours. Subsequently, the kinetics of the relaxation to the thermodynamically stable phase at the same temperature is followed, that is exceptionally slow on the order of hours or days.

P-28

The glue that holds the Gram-negative Outer Membrane together and keeps antibiotics out

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The outer membrane (OM) of Gram-negative bacteria is an unusual asymmetric bilayer with an external monolayer of lipopolysaccharide (LPS) and an inner layer of phospholipids. The LPS layer is rigid and stabilized by divalent cation cross-links between phosphate groups on the core oligosaccharide regions. This means that the OM is robust and highly impermeable to toxins and antibiotics. During their biogenesis, OM proteins (OMPs), which function as transporters and receptors, must integrate into this ordered monolayer while preserving its impermeability. Recently we revealed the specific interactions between the trimeric porins of Enterobacteriaceae and LPS and showed that these interactions are essential for trimeric porin folding in the outer membrane. The high resolution structure of the porin trimer displays three binding sites of which one contains a calcium ion. This is reminiscent of the interactions which maintain the structure of pure LPS monolayers. Furthermore the LPS-protein interactions display complex polar interactions that are unlike previous LPS binding sites. The resolution of this well resolved interface gives clues as to the stabilising forces at work in the dynamic lipid-rich regions of the membrane.

Posters

– 1. Multiscale biophysics of membranes –

P-29

Impact of cholesterol on lipid-protein interactions revealed by quasielastic neutron scatteringL. Lautner¹, W. Lohstroh², T. Seydel³, S. A. Kirsch⁴, R. Böckmann⁴, T. Unruh¹¹Chair for Crystallography and Structural Physics, FAU Erlangen-Nürnberg, Germany; ²Heinz Maier-Leibnitz Zentrum (MLZ), TU München, Germany; ³Institut Laue-Langevin (ILL), Germany; ⁴Computational Biology, FAU Erlangen-Nürnberg, Germany

Quasielastic neutron scattering (QENS) is a powerful tool to analyse dynamics in biological membranes. These dynamics depend critically on the interplay between the major parts of membrane components – lipids, proteins and sterols – which play an essential role in maintaining a variety of cellular functions. Which role plays the protein concentration on membrane dynamics? What is the impact of cholesterol on lipid-protein-interactions? These questions were addressed in recent QENS experiments at the backscattering instrument IN16B (ILL, Grenoble) and the time-of-flight spectrometer TOFTOF (MLZ, Garching). Thereby, DMPC lipid vesicles with respectively 0, 2, and 6 mol% of a transmembrane sequence of the receptor protein transferrin (TFRC) were measured. Moreover, different cholesterol concentrations were added to the pure DMPC vesicles and the vesicles with 2 mol% of TFRC. The results indicate a restricted lipid mobility for the lipid-protein vesicles compared to the pure lipid vesicles for all protein concentrations in the fluid-crystalline phase. Moreover, diffusion coefficients for both the lipids and proteins could be determined and the influence of cholesterol on the lipid-protein-interactions was analysed.

P-31

Membrane interaction of the glycosyltransferase WaaGJ. Liebau, B. Fu, C. Brown, L. Mäler
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Monotopic glycosyltransferases (GTs) interact permanently with membranes via electrostatic interactions mainly of the N-terminal domain. However, membrane affinities of GTs are largely unknown. The glycosyltransferase WaaG that is involved in the synthesis of lipopolysaccharides (LPS) in Gram-negative bacteria was previously categorized as a monotopic GT based on its subcellular localization. We analyzed the binding of WaaG to membranes by stopped-flow fluorescence and NMR diffusion experiments. We find that electrostatic interactions are required to bind WaaG to membranes while mere hydrophobic interactions are not sufficient. WaaG senses the membrane's surface charge density but shows no preferential binding to specific anionic lipids. The membrane binding is weaker than expected for monotopic GTs but similar to peripheral GTs. Therefore, WaaG may be a peripheral GT and this could be of functional relevance *in vivo* since LPS synthesis occurs only when WaaG is membrane bound. NMR diffusion experiments show that the donor nucleotide sugar UDP-glucose does not bind to soluble or membrane-bound WaaG in the absence of the nascent LPS acceptor molecule. We assume that binding of nascent LPS to WaaG is required for UDP-glucose binding.

P-30

Illuminating the spatio-temporal dynamics of lipopolysaccharide in the bacterial outer membraneR. M. Leaman, R. J. Spears, M. A. Fascione, D. O. Pushkin, M. C. Coles, C. G. Baumann
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The outer membrane (OM) of a Gram-negative bacterium is a barrier that protects the cell from environmental challenges. The cell must turnover its OM components to adapt to new environmental niches and to avoid destruction by the host's immune system. Recently, it was discovered that OM proteins are inserted at discrete locations and that they undergo confined diffusion^{1,2}. This leads to protein 'islands' in the OM that appear at mid-cell and only move during cell growth. By contrast, little is known about the spatio-temporal dynamics of lipopolysaccharide (LPS), a major component of the OM. Previous reports on LPS diffusion are contradictory, as both unrestricted and confined behaviour was observed. We probed the insertion and diffusion of LPS using metabolic incorporation of a sugar analogue and site-specific bio-orthogonal chemical labeling with a fluorescent dye³. Using fluorescence microscopy, we observed that LPS does not diffuse freely in the OM and is inserted at discrete locations during cell growth.

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P-32

Charge-dependent membrane fusion in a pure lipid systemR. B. Lira^{1,2}, T. Robinson², R. Dimova², K. A. Riske¹¹Departamento de Biofísica, Universidade Federal de São Paulo, Brazil; ²Department of Theory and Biosystems, Max Planck Institute of Colloids and Interfaces, Potsdam, Brazil

Membrane fusion is a ubiquitous process in biology and is a prerequisite for many intracellular delivery protocols relying on the use of liposomes as drug carriers. Here we investigate in detail the process of membrane fusion and the role of opposite charges in a pure lipid system based on cationic liposomes composed of 1:1 DOTAP:DOPE with 5 mol% of a fluorescent lipid and (anionic) giant unilamellar vesicles (GUVs) composed of different POPC:POPG molar ratios. By using a set of optical microscopy-based methods, we show that liposomes strongly dock to GUVs of pure POPC or low POPG fraction (up to 10 mol%), in a process mainly associated with hemifusion and membrane tension increase, commonly leading to GUV rupture. On the other hand, docked LUVs quickly and efficiently fuse with negative GUVs of POPG fractions at or above 20 mol%, resulting in GUV area increase in a charged-dependent manner. Importantly, both hemifusion and full fusion are leakage-free. Fusion efficiency is quantified from the lipid transfer from liposomes to GUVs upon fusion by fluorescence resonance energy transfer (FRET) efficiency and allow for the determination of the final composition of single GUVs after fusion. We can conclude that fusion is driven by membrane charge and occurs up to charge-neutralization of the acceptor GUV system. Financial support: FAPESP and MaxSynBio.

Posters

– 1. Multiscale biophysics of membranes –

P-33

Charged liposomes of varied lipid composition in the presence of polylysines: quantitative analysis

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According to our previously published data [1] electrokinetic measurements reveals the irreversible adsorption of polylysines at the surface of liposomes composed from neutral (PC) and anionic (CL) phospholipids. Zeta-potential of liposomes of varied composition was measured in the presence of polypeptide of different molecular weight and detect the region of a linear growth in respect of polylysine content in the suspension and the saturation level. We develop a theoretical model with two adjustable parameters – thickness h of polylysine layer at the membrane surface and fraction, β , of the area occupied by polymer. The model presents polypeptides as solid positively charged blocks. Fitting of the data shows the value of h increased with the polymer length and parameter β proportional to the total surface charge of the membrane. The total surface coverage by polymers decreases with the number of lysine bases in polylysine chain, and these dependences vanish for a long macromolecule. Fitting results correlate with the related atomic force data. More realistic geometry of lipid-polymer clusters and ion distribution over its body are expected from MD analysis of the system.

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P-35

Behaviour of ceramide in mitochondria-mimicking membranes

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Ceramides form ordered domains in otherwise fluid membrane environments, which can affect both lateral and transmembrane movement of lipids and membrane proteins, and can ultimately lead to loss of membrane integrity and possibly membrane permeabilization. Although ceramide content of cells and mitochondria is kept very low during physiological conditions, the accumulation of ceramide in the mitochondria has been linked to apoptosis. Ceramide aggregation is suggested to induce leaking of the mitochondrial membrane, although it is unknown if this triggers apoptosis or is a consequence of it. As the mitochondrial outer membrane mostly consists of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and cardiolipin in smaller amounts, we have studied how palmitoyl ceramide interacts with these lipids, and how its lateral segregation is affected by them. Among the methods to study ceramide lateral segregation, we used time resolved fluorescence spectroscopy as well as differential scanning calorimetry and NMR.

P-34

Polystyrene incorporated within lipid membrane disrupts bilayer's phase transition

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Recently, serious concerns regarding accumulation of polystyrene and similar polymeric nanoparticles in eukaryotic cells have been raised due to the growing plastic waste. Although previous studies have shown that nanoscopic sized polymers can accumulate in cells, the effects on cell metabolism is far from being understood, and the fundamental mechanisms of their interaction with the different cell components are yet to be addressed. Molecular dynamics simulations indicate significant effects on lipid phase behavior induced by polystyrene, suggesting potential hazardous implications for the plasma membrane. However, experimental results performed on cells (model) membranes are still scarce.

We focus here on understanding how incorporated polystyrene affects the phase behavior of model membranes. We combined differential scanning calorimetry (DSC) and analysis of Laurdan emission spectra to obtain information on the bilayer structure; we then correlated the changes in the membrane with macroscopic alterations of phase transition using Laser Scanning Confocal Microscopy on giant unilamellar vesicles (GUVs). Our results show that polystyrene disrupts the phase behavior of lipid membranes modifying the thermodynamics of the transition through a spatial modulation of lipid composition.

P-36

Nonuniqueness of local stress of three-body potentials in molecular simulations

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Microscopic stress fields are widely used in molecular simulations to understand mechanical behavior. Recently, decomposition methods of multibody forces to central force pairs between the interacting particles have been proposed [1,2]. Unlike the conventional force decomposition method which is called Goetz Lipowsky decomposition, the stress tensor calculated using this method satisfy the conservation of both translational and angular momentum.

Here, we introduce a force center of a three-body potential and propose different force decompositions that also satisfy the conservation of both translational and angular momentum [3]. We compare the force decompositions by stress-distribution magnitude and discuss their difference in the stress profile of a bilayer membrane by using coarse-grained and atomistic molecular dynamics simulations. We demonstrate that the stress profile strongly depends on the force decompositions.

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Posters

– 1. Multiscale biophysics of membranes –

P-37

Interaction of bile salts with lipid bilayers: a multiscale molecular dynamics study

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Bile salts (BS) are biosurfactants synthesized in the liver and secreted into the intestinal lumen where they solubilize cholesterol and other hydrophobic compounds facilitating their gastrointestinal absorption. Interaction of BS with lipid membranes is an important step in both processes. In this work, we used molecular dynamics simulations to address the interactions of cholate, deoxycholate and chenodeoxycholate, as well as their glycine conjugates with POPC bilayers. From atomistic simulations with low to moderate BS:phospholipid ratio, BS properties such as location and orientation inside the bilayer, and specific interactions with water and host lipid (hydrogen bonding and ion-pair formation) were studied in detail. Membrane properties were also investigated to obtain information on the degree of perturbation induced by the different BS, which was highest for cholate. Subsequently, we carried out 10- μ s coarse-grained simulations of this BS in POPC at higher BS:phospholipid ratios, to investigate the occurrence of BS aggregation, translocation, insertion and desorption events in this time scale. The results will be described and related to a recent experimental study (Coreta-Gomes et al., Langmuir, 2015, 31:9097). Funding by FCT (Portugal), project n^o 007630 UID/QUI/00313/2013 (co-funded by COMPETE2020-UE) is acknowledged.

P-39

Interaction of fengycin C biosurfactant with phosphatidylcholine model membranes

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Bacillus subtilis strain EA-CB0015 produces iturin A, fengycin C and surfactin, and its cell free supernatants inhibit *M. fijiensis* cellular growth, a pathogen which causes black Sigatoka disease in banana. Iturin A and fengycin C inhibit mycelial growth and ascospore germination, whereas surfactin is not effective. On the hypothesis that the antifungal action of the lipopeptides is linked to their interaction with biological membranes, a detailed biophysical study on the interaction of a new isoform of fengycin C with model DPPC membranes has been carried out. Differential scanning calorimetry shows that fengycin C alters the thermotropic phase transitions of DPPC, and is laterally segregated in the fluid bilayer forming domains. Fluorescent probe polarization measurements show that fengycin C affects the lipid/water interface of the membrane, which is concomitant with a strong dehydration of the polar region of DPPC, as shown by FTIR. Fengycin-rich domains, where the surrounding DPPC molecules are highly dehydrated, may well constitute sites of membrane permeabilization leading to a leaky target membrane. These results are a solid support to explain the membrane perturbing action of fengycin, which has been related to its antifungal activity.

P-38

Membrane Shape Transformation Induced by Banana-Shaped Proteins

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In living cells, morphology of biomembranes is regulated by various proteins. Many of these proteins contain a banana-shaped binding module called BAR (Bin-Amphiphysin-Rvs) domain. We have studied how anisotropic spontaneous curvatures of banana-shaped protein rod induce effective interaction between the proteins and change membrane shapes by using implicit-solvent meshless membrane simulations [1-5].

The self-assembly of the rods is divided to two directional assemblies at the low rod density [1] and polyhedral and high-genus vesicles are formed at the high density [2,3]. A small spontaneous curvature perpendicular to the rod can remarkably alter the tubulation dynamics at high rod density whereas minor effects are only obtained at low density [4]. Two types of the protein rods with opposite rod curvatures cooperatively induce straight bumps and stripe structures [5].

[1] H. Noguchi, *EPL* **108**, 48001 (2014): [2] H. Noguchi, *J. Chem. Phys.* **143**, 243109 (2015): [3] H. Noguchi, *Phys. Rev. E* **93**, 052404 (2016): [4] H. Noguchi, *Sci. Rep.* **6**, 20935 (2016): [5] H. Noguchi and J.-B. Fournier, arXiv:1702.04107.

P-40

Intrinsic lipid curvature and membrane charge modulates synergistic activity of magainin peptides

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Mixtures of the antimicrobial frog peptides magainin 2 and PGLa are well-known for their synergistic killing of bacteria. We correlated the permeabilizing efficacies of the two magainins found for vesicles mimicking either bacterial or mammalian plasma membranes with (i) the intrinsic lipid curvature, J_0 , as determined by small-angle X-ray scattering and (ii) the surface charge of the membrane. Synergism was only observed in phosphatidylethanolamine (PE)/phosphatidylglycerol (PG) mimics of bacterial membranes with large negative J_0 . Replacing PE by phosphatidylcholine (PC) or appropriate amounts of lyso-PE yielded bilayers of the same net charge, but $J_0 \sim 0$. In these systems both peptides were highly active, but did not act synergistically. Charge neutral lipid bilayers of PC ($J_0 \sim 0$) in turn exhibited little leakage upon addition of the peptides, which was even further decreased when shifting J_0 to negative values by addition of cholesterol. Our results allude to the central roles of stored membrane curvature energies and bilayer charge distribution for antimicrobial peptide activity, emphasizing the delicate choice of lipid-only mimics of natural membranes.

Posters

– 1. Multiscale biophysics of membranes –

P-41

Iron-mediated interaction of A53T alpha synuclein with artificial lipid bilayers

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Alpha Synuclein (AS) is a cytosolic neuronal protein associated with neurodegenerative diseases, called synucleinopathies. AS is able to bind cellular membranes promoting protein aggregation and toxicity. Recent findings suggest that brain iron accumulation correlates with disease stage and severity.

We correlated iron content with AS propensity to form aggregates and to interact with biological membranes. By AFM, we investigated the role of iron (II) in the interaction of the mutated A53T AS with artificial lipid bilayers composed by DOPC, sphingomyelin and cholesterol, mimicking lipid raft domains (LRs).

Iron induces the formation of globular protein aggregates which preferentially interact with LR. On the contrary, in absence of iron, AS interact primary with the DOPC leading to the formation of defects without any aggregation. We also induced in vitro iron-mediated protein oligomers which form structures similar to those caused by monomers co-incubated with iron, but the new structures appear bigger and more irregular in shape, probably due to the stronger activity of oligomers.

Our work showed that iron has a huge effect in promoting aggregation of AS on lipid bilayers, highlighting some of the molecular mechanisms which are at the basis of synucleinopathies development.

P-43

The nanoscale dynamics of fluid lipid bilayers is specifically modulated by ions

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Ions are known to affect the mechanical properties of biological membranes, but their impact on the membranes' dynamics at the nanoscale is still largely unexplored. Here, we use atomic force microscopy (AFM) to systematically examined the effect of Na⁺, K⁺, Ca²⁺ and Tris on the structure and dynamics of supported 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) bilayers in solution. We show that the ions can induce local molecular ordering of otherwise fluid membrane over a characteristic ~20 nm lengthscale. The effect is strongly ion-specific, and exacerbated when a mechanical stress is applied to the membrane. The results indicate that calcium and Tris have opposite effects in respectively promoting and reducing nano-mechanical ordering of the lipid molecules. FRAP measurements show that the tendency for the bilayer to form nano-ordered domains is inversely proportional to the lipids' diffusion coefficient within the bilayer. The existence of ion-induced local order can spatially modulate the adsorption of biomolecules to the membrane; here illustrated with the membrane-penetrating antimicrobial peptide Temporin L. Our results suggest that ionic effects can have a significant impact on biological membranes' function by modulating nanoscale structure and dynamics.

P-42

A lipid molecule containing fluid isotropic and solid segments anchored in a solid lamella

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We herein investigate composition and molecular dynamics of a minor fluid fraction in model lipid systems of stratum corneum (SC), the outermost layer of the skin, induced by ceramide esterified omega-hydroxy sphingosine (CER EOS) linoleate, which is crucial for the formation of a SC lamellar structure. It is shown that the fluid lipid fraction consists of segments in the middle and terminal of the acyl-chains and segments of the conjugated C=C double bonds of the linoleate moiety that undergo isotropic reorientation on time-scales shorter than 10 ns, as previously observed in intact SC and other SC lipid models, and the self-diffusion of the conjugated C=C chain segments is extraordinary slow (~10⁻¹⁴ m² s⁻¹). We therefore envision an isotropic fraction consisting of dangling EOS linoleate tails embedded and anchored in the solid lamellar structure. The findings provide further insight into the molecular arrangement in the SC lipid matrix and the role of CER EOS linoleate in the SC barrier function.

P-44

Dynamics of biological membranes - Quasi elastic neutron scattering studies of model membranes

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Real biological membranes are complex multi-component systems which are not easily subjected to systematic investigations, although e.g. the dynamics of light-induced structural reorganizations of thylakoid membranes in chloroplast organelles have been studied using time-resolved small-angle neutron scattering [1]. The complementary approach to the study of real membranes is the study of phospholipid model membranes with full control of lipid species together with the type and concentration of any other molecule embedded in the membrane and with a certain control over the large scale membrane organization. Adding a series of 1-alcohols to DMPC membranes changes membrane structural properties such as bilayer thickness and lipid packing. We have performed quasi elastic neutron scattering in the picosecond to nanosecond time range in order to investigate the influence of membrane architecture and membrane doping on the nanoscopic dynamics of phospholipids in multilamellar and unilamellar vesicles in excess water. These results together with structural characterization will be discussed.

[1] D. Posselt *et al*, Biochim.Biophys.Acta **1817** (2012) 1220; G. Nagy *et al*, Eur. Phys. J. E 36 (2013) 69

Posters

– 1. Multiscale biophysics of membranes –

P-45

Diffusion of lipids and GPI-anchored proteins in the plasma membrane and actin free vesiclesF. Schneider¹, E. Sezgin¹, D. Waithe², C. Eggeling¹¹MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK; ²Wolfson Imaging Centre, Weatherall Institute of Molecular Medicine, University of Oxford, UK

The plasma membrane is known to be highly complex and heterogeneously organised on nano-scales. The diffusion of proteins and lipids in the cellular membrane is an important measure for this membrane heterogeneity and transit interactions of molecules. In this study, we use super-resolution STED-microscopy in combination with fluorescence correlation spectroscopy (STED-FCS) to investigate the diffusional properties of lipid probes and GPI-anchored proteins (GPI-APs) in the plasma membrane and compare our findings to the diffusional behaviour in actin cytoskeleton free cell-derived plasma membrane vesicles (GPMVs). In the living cells, we find a variety of different diffusion characteristics (such as hop and confined diffusion modes) but this hindered diffusion is mostly abolished in GPMVs for phospho- and sphingolipids as well as for GPI-anchored proteins. Only for the ganglioside GM1, domain like diffusion persists in the model membrane system. This study underlines the strong influence of the cortical actin cytoskeleton on the plasma membrane organisation but also highlights actin and energy independent domain formation of certain molecules in the membrane.

P-47

A tale of actin and lipid antigen presentation - A nano tango

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The organization and dynamics of cell surface proteins such as receptors are important for cellular signalling. In this context, the essential role of the actin cytoskeleton is immensely appreciated. Here, we investigate the correlation between antigen presentation in antigen-presenting cells (APCs) and its actin cytoskeleton organization. Antigen presentation is a hallmark feature of adaptive immune response, where foreign antigens (e.g. from bacteria or viruses) are presented to T cells for generation of appropriate immune responses. Using advanced optical microscopy, specifically line-scan Fluorescence Correlation Spectroscopy (lsFCS) we investigated the interplay between the actin cytoskeleton organization and diffusion dynamics of the lipid-antigen presenting protein CD1d. Using specific drugs affecting distinct actin assembly pathways, our lsFCS diffusion experiments made a unique revelation suggesting a crucial role of the cytoskeletal machinery in APCs in fine-tuning lipid antigen presentation. Especially, the finer cortical actin network underneath the plasma membrane was found to be a critical determinant factor in the diffusion of CD1d.

P-46

Lipid domains in confined geometry – How boundaries define domain size and mobilityO. M. Schütte¹, I. P. Mey¹, J. Enderlein², F. Savić³, B. Geil³, A. Janshoff³, C. Steinem¹¹Institute of Organic and Biomolecular Chemistry, University of Goettingen, Germany; ²Third Institute of Physics, University of Goettingen, Germany; ³Institute of Physical Chemistry, University of Goettingen, Germany

In the plasma membrane of eukaryotic cells, proteins and lipids are organized in clusters, the latter one often called lipid domains or ‘lipid rafts’. Recent findings highlight the dynamic nature of such domains and the key role of the cytoskeleton meshwork in stabilizing lipid domains. In this study, we used gold-covered functionalized porous silicon substrates with different pore radii serving as a static meshwork to modulate the size of lipid domains in phase-separated continuous pore-spanning membranes (PSMs) mimicking the plasma membrane. Fluorescence video microscopy revealed two types of liquid-ordered (l_o) domains in the freestanding parts of the PSMs: i) immobile domains that were attached to the pore rims and ii) mobile round shaped l_o domains. Analysis of the diffusion of the mobile l_o domains showed that the domains’ diffusion constants are slowed down by orders of magnitude compared to the unrestricted case due to the confinement of the PSM governed by both, friction in the bilayer and coupling to the aqueous phase. This strongly reduced domain diffusion within corralled areas can have great implications for the formation and confinement of signaling platforms, which need to be mobile but localized to certain membrane areas.

P-48

Importance of membrane curvature near hole edges in plasma membrane repairA. C. Simonsen¹, T. L. Boye², J. Nylandstedt²¹University of Southern Denmark, Department of Physics, Chemistry and Pharmacy, Section for Soft Matter and Biological Physics, Denmark; ²Danish Cancer Society Research Center, Membrane Integrity Group, Unit for Cell Death and Metabolism, Denmark

Maintaining integrity of the plasma membrane is essential for cell life. Thus, efficient cell membrane repair mechanisms are crucial for handling membrane disruptions resulting from perturbations of eukaryotic cells. Yet, the underlying molecular details of plasma membrane repair are not understood. Plasma membrane injury followed by Ca^{2+} influx, activates the recruitment of Annexins to membrane wound edges. In cells, Annexin A4 binding promotes repair of lesions generated by e.g. local laser treatment. In planar model membranes, we show that curvature stress induced by annexin-binding leads to roll-up of the membrane as initiated from free membrane edges. The observation of rolling identifies plasma membrane curvature near hole-edges as a potential key event in the plasma membrane repair (PMR) process. In cells, we propose that such curvature may lead to the formation of a characteristic neck structure around holes as a first step in the membrane repair process. Here we discuss the phenomenon of annexin-induced membrane curvature near edges, including results obtained from model membranes, cells studies and theoretical modeling. Together they provide an enhanced mechanistic insight into the role of annexins for initiating membrane repair.

Posters

– 1. Multiscale biophysics of membranes –

P-49

Flax phenolic compounds: elucidation of their mechanism to prevent lipid peroxidation

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Polyunsaturated fatty acids are essential in living organisms [1]. However, they are extremely sensitive to the damages caused by reactive species. Indeed, the oxidation of lipids has been reported to be involved in the early stages of several diseases [2] and the degradation of food [3]. To prevent lipid peroxidation, various antioxidants such as polyphenols have been used. The culture of flax is particularly developed in France and the seeds are mainly used to extract oils. However, they also contain an important amount of phenolic compounds [4]. Thus, to valorize the whole plant, we extracted polyphenols from flax seeds and studied their influence on lipid oxidation by using biomimetic lipid systems. We showed that lignans are more efficient than hydroxycinnamic acids to prevent lipid oxidation. Interestingly, the comparison of the different lignans antioxidant efficiencies revealed important discrepancies, showing the relationship between the membrane affinity and the antioxidant efficiency. [1] M. Rodríguez-Cruz *et al.*, Rev. Investig. Clínica; 57 (2005). [2] X. Zhang *et al.*, Eur. Rev. Med. Pharmacol. Sci. 18 (2014). [3] D. McClements, E. Decker, J. Food Sci. 65 (2000). [4] A.I. Smeds *et al.*, J. Agric. Food Chem. 55 (2007).

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P-51

Computational study of oxygen transport across domains of the membrane of the eye lens fibre cells

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Molecular oxygen (O₂) transport across biomembranes is of great importance in many cellular processes. The O₂ permeability coefficient across different membrane domains can be obtained using electron paramagnetic resonance spin-labeling (EPR-SL) methods that measure oxygen diffusion-concentration product as a function of the bilayer depth. Unfortunately, using EPR-SL neither the coefficient for the membrane pure cholesterol (Chol) domain, called CBD, nor the separate diffusion and concentration profiles across the membrane can be obtained. In contrast, molecular dynamics (MD) simulation enables calculation of both O₂ permeation across the CBDs and separate diffusion coefficient and concentration profiles across any membrane domain. In this study, three computer models were built and MD simulated to calculate separately O₂ diffusion and concentration profiles and permeation across purported domains of the eye lens membrane: the CBD and the bulk 1:1 molar ratio POPC-Chol bilayer (Chol saturated membrane, in which CBD is embedded); and across a reference POPC bilayer. Results obtained with MD simulations for POPC-Chol and POPC bilayers agree well with the EPR-SL data and indicate that CBD is a barrier to O₂ transport in the eye lens cell.

P-50

Stiffness of natural extra-cellular vesicles is governed by membrane protein content

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Extracellular vesicles (EVs) are important mediators of intercellular communication, being involved both in maintaining normal physiology as well as spreading of a wide range of diseases. In order to successfully deliver their cargo, EVs need to be taken up by the target cells. Several studies suggest that successful cellular uptake of nanoparticles is affected by their mechanical properties. We propose that mechanical properties of EVs are important with respect to their function. We study mechanics of vesicles from red blood cells (RBC), both healthy and malaria parasite infected. Moreover, we examine the effect of cell temperature treatment on the mechanical properties of the secreted vesicles. To do so we perform a detailed AFM force spectroscopy study and analyze our results using a Helfrich-model based theoretical framework to estimate the bending modulus of different vesicle populations. By simultaneously performing a systematic analysis of EV protein and lipid composition, we find that bending modulus values are significantly decreased upon increase in EV membrane protein content. Our results can provide better understanding of EVs function and new insights into the vesiculation process in health and disease.

P-52

Oxidized phospholipids induce formation of inter-leaflet coupled nanodomains in giant lipid vesicles

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Oxidized phospholipids (OxPLs) are products of irreversible oxidation of unsaturated fatty acid side chains. Although they are associated with several inflammatory diseases such as atherosclerosis and neurodegenerative diseases like Parkinson's and Alzheimer's disease their exact role in living organism is not fully understood. In this work we investigated the impact of two different types of OxPLs (1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC)) on lipid nanodomains found in DOPC, cholesterol and sphingomyelin lipid mixtures. By applying a unique approach based on Förster resonance energy transfer (FRET) sizes and concentrations of nanodomains were determined. It follows from comparison of different fitting models of FRET that the nanodomains are coupled in between leaflets of the lipid bilayer. Properties of OxPLs rich nanodomains were characterized by solid-state NMR and were compared to those without OxPLs. In general, profound effects were observed in the presence of both OxPLs.

Posters

– 1. Multiscale biophysics of membranes –

P-53

Lipid bilayer-coated silica beads in membrane fusion assays

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Membrane fusion is a key process in life, and therefore widely studied in vivo as well as in model systems. Our group employs model systems involving membrane-coated silica microspheres. Our techniques can provide data on several particles within a single experiment, employing long-time fluorescence microscopy and FRAP. In fused pairs of beads, it was shown by simulation as well as experiments that this contact zone is likely so small that it strongly obstructs lipid diffusion across the fusion spot, as compared to diffusion within a single bead. The same is the case for the fusion of membrane-coated beads with a planar membrane. In this new assay, fusion behaviour of different sphere radii can be compared, simultaneously with fluorophore diffusion across the fusion contact zone.

P-54

Membrane fusion accelerated by normal forces and elevated in-plane tension

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Fusion of lipid bilayers is usually prevented by large energy barriers arising from removal of the hydration shell, formation of highly curved structures and eventually fusion pore widening. Here, we measured the force-dependent lifetime of fusion intermediates by using membrane-coated silica spheres in contact with supported lipid bilayers. Analysis of time traces obtained from force clamp experiments allowed us to unequivocally assign different membrane states during the SNARE-mediated fusion process. We identified the removal of the hydration shell to be the main energy barrier. Since external normal forces are absent in native systems, we also addressed the impact of orthogonal forces. Therefore, isolated patches of planar bilayers were formed from giant unilamellar vesicles and deposited on a dilatable PDMS sheet. We found that fusion efficiency increases considerably with lateral tension and identified a threshold tension at which fusion is boosted. Taken together, the parameter space of fusion has been widened to increase our understanding how fusion is accelerated in living systems.

P-55

The main lipid in the thylakoid membrane stabilizes the Light Harvesting Complex IIH. Witt¹, D. Seiwert², A. Janshoff¹, H. Paulsen²¹University of Göttingen, Germany; ²Johannes Gutenberg University Mainz, Germany

The light-harvesting complex in the photosystem II of green plants (LHCII) is the most abundant membrane protein on earth. It is embedded in the thylakoid membrane, which is notorious for containing a high percentage of 50% of the non-bilayer lipid monogalactosyldiacylglycerole (MGDG). The conical shape of MGDG is presumably compensated by the hourglass shaped trimeric LHCII, which was found to be essential for the integration of large fractions of MGDG into lamellar membranes.

Here we use single-molecule force spectroscopy to understand the interaction between LHCII and its lipid matrix by unfolding the trimeric protein from membranes composed of different thylakoid lipids. We observed a substantial increased mechanical stability of LHCII in membranes containing MGDG, which we attribute to their sterical interplay.

The role of non-bilayer lipids in cellular membranes is still under debate. It is mostly assumed to impact the membrane structure and topology by mediating spontaneous curvature. The mutual stabilization between LHCII and MGDG observed here might contribute to the understanding of the widespread occurrence of non-bilayer lipids in biological membranes.

P-56

Studies of interactions of 5-n-alkylresorcinols with lipid membranesP. Zawilska¹, K. Cieřlik-Boczula²¹Faculty of Biotechnology, University of Wrocław, Joliot-Curie 14a, 50-383 Wrocław, Poland; ²Faculty of Chemistry, University of Wrocław, Joliot-Curie 14, 50-383 Wrocław, Poland

5-n-alkylresorcinols (ARs) are amphiphilic compounds that can alter the bilayers' biophysical properties. ARs demonstrated a stabilizing effect on lipid bilayers by making the bilayers less permeable. The aim of this study was to understand how AR homologs (C15–C25) affect biophysical properties of phospholipid membranes. For characterization of the AR-induced alterations to membranes, Laurdan fluorescence and attenuated total reflectance-infrared spectroscopy (ATR-IR) were used. Dipalmitoylphosphatidylcholine (DPPC)-pure or cholesterol (Chol)-enriched were used as model membranes. Chain length-dependent changes in DPPC:AR and DPPC:Chol:AR membranes suggest strong AR–DPPC and AR–DPPC:Chol membrane interactions. Incorporation of ARs into membranes led to a shift in the gel-liquid crystalline phase transition of ARs-mixed bilayer toward higher temperatures. With an additional increase in the alkyl-chain length and concentration of doped compounds, we observed lower cooperativity of the chain-melting phase transition, which is a sign of increased stiffness within the lipid acyl chains. Furthermore, ARs decreased lipid headgroup hydration. We also demonstrated that -OH groups of AR molecules form hydrogen bonds with the DPPC lipid phosphate groups; this may explain the stabilizing effect of ARs on bilayers.

Posters

– 2. Molecular machinery –

P-57 (O-19)

Structural dynamics of the 70S ribosome during translocation monitored by single-molecule FRET

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Ribosome dynamics play an important role in translation. The rotation of the ribosomal subunits relative to one another is essential for tRNA-mRNA translocation. We monitor subunit rotation relative to peptide bond formation and translocation using ensemble kinetics and single-molecule FRET. We find that large-scale movements of the ribosome are intrinsically rapid and gated by its ligands such as EF-G and tRNA.

The coupled translocation of tRNA and mRNA through the ribosome includes step-wise movements of the tRNAs. Structural work has visualized intermediates of translocation induced by EF-G with tRNAs trapped in chimeric states with respect to 30S and 50S ribosomal subunits. The functional role of the chimeric states is not known. We follow the formation of translocation intermediates by single-molecule FRET. Using EF-G mutants, a non-hydrolysable GTP analogue, and the antibiotic fusidic acid, we interfere with either translocation or EF-G release from the ribosome and identify several rapidly interconverting chimeric tRNA states on the reaction pathway. Our data illustrates that the engagement of EF-G alters the energetics of translocation towards a flat energy landscape, thereby promoting forward tRNA movement.

P-59 (O-21)

Single-molecule dissection of cytoplasmic dynein force sensing

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Cytoskeletal motor protein motility requires coordination of ATPase and filament-binding cycles. Mechanical tension, which arises from both external and intramolecular forces, regulates motor stepping. In cytoplasmic dynein—a unique AAA+ ATPase with six linked AAA+ domains (AAA1-6), a coiled-coil stalk that connects the AAA+ ring and microtubule (MT)-binding domains, and a dimerizing tail domain—applied tension affects microtubule (MT)-binding strength anisotropically, with backward tension inducing stronger binding. Using optical tweezers, mutagenesis, and chemical cross-linking, we elucidate the underlying molecular mechanisms of this behavior, showing that either preventing relative motion of the dynein stalk helices or deleting the coiled coil ‘strut’ that emerges from AAA5 and contacts the stalk eliminate tension-based regulation of MT-binding. In addition, linker docking/undocking to and from AAA5 controls tension-induced stalk reconfiguration. Thus, tension alters dynein’s MT-binding strength by inducing sliding of the stalk helices, mediated by the strut and linker.

P-58 (O-20)

Stepping motion and chemo-mechanical coupling of chitinase resolved by single-molecule analysis

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Serratia marcescens chitinase A (SmChiA) is a monomeric linear molecular motor moving on and hydrolyzing crystalline chitin processively. Here we directly resolved steps and pauses in the motion of SmChiA with high-resolution single-molecule imaging analysis. By using total internal reflection dark-field microscopy and 40-nm gold nanoparticle as a low-load probe, movement of SmChiA was observed at 1,000–2,000 frames/s with 0.3 nm localization precision. The step sizes were 1.1 nm and -1.3 nm for forward and backward steps, respectively, consistent with the length of the product, chitobiose (~1 nm). The ratio of forward to backward steps was 2.9, corresponding to the energy difference of 1.1 $k_B T$. Frequent backward steps and low energy difference indicate that SmChiA operate as the Brownian ratchet. Furthermore, detailed analysis of the distribution of pause duration revealed that the rate-limiting step of chemo-mechanical coupling of SmChiA is the decrystallization of single polymer chain from the crystalline chitin, not bond cleavage and product release. These results give us important insights to engineer non-natural chitinases which show better performances than the natural ones.

P-60

Variation in stride length of myosin-5a along actin shown by interferometric scattering microscopy

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Myosin-5a takes 36 nm steps, corresponding to every 13th actin subunit along the F-actin helix. EM studies have shown that in addition to the expected 13 subunit steps, myosin also takes shorter and longer (11 and 15 subunit) steps. Characterising this behaviour with optical trapping or FIONA however, have only exhibited a broad distribution of step lengths with a mean at 36 nm, leading to the conclusion that all steps correspond to 36 nm, with a broadening resulting from experimental uncertainty.

To address this inconsistency, we took advantage of the unique sub-nm localisation precision of iSCAT. We tracked myosin with a 20 nm gold particle bound to the motor domain of just one of the two heads, which allowed us to observe the strides taken by one motor as it detaches, moves past the attached motor and rebinds in front of it. Here, the expected step size of the labelled head would be 72 nm corresponding to 26 actin subunits. Detailed analysis of the iSCAT traces revealed a family of peaks separated by intervals of 5.5 nm, corresponding to strides spanning 22, 24, 26, 28 and 30 subunits as predicted from the F-actin structure. Our results suggest how the motor navigates obstacles, which may help effective movement of myosin through the crowded environment of the cytoplasm.

Posters

– 2. Molecular machinery –

P-61

Hierarchical construction of molecular machines

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In a bottom-up fashion we aim to organize functional devices weaved in the form of hierarchical networks from the nanoscale to collectively assembled macroscopic building blocks. This “lego” organization is managed so that the efficacy of substitutions of any construct at any scale for superior properties may readily be assessed. We, as the MIDST Lab (<http://midst.sabanciuniv.edu/>), by utilizing biomimetics not only on the macro, but on all scales, have found ways to achieve this ambition. Non-synonymous single nucleotide polymorphisms giving rise to amino acid substitutions have shown to alter drastically function of proteins. We have developed simple, yet effective, methods to characterize the consequences of those substitutions either on the conformations, or on the electrostatic distributions and, therefore, on the dynamics and function of biological macromolecules. By changing a residue's physical/chemical characteristics, or the micro-environment including the temperature and the ionic strength of the surrounding water body, we have effectively demonstrated and confirmed with experimental studies that the turnover rate of an enzyme or the time scale of ligand release may be altered in a controlled manner. We discuss the future prospects of the developed understanding.

P-63

Structural dynamics in the myosin 7a single α -helix domain

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Single α -helical domains (SAHs) are found in many different proteins, and are highly stable across a range of pH and ionic strength conditions. Rich in charged amino acid residues (Glu (E), Lys (K) and Arg (R)), their stability arises from a network of stabilising ionic interactions. The best-studied examples of SAHs are those from myosin motor proteins in which they form part of the lever. Using CD and NMR, we have investigated the structure and flexibility of the SAH domain from myosin 7a, which is 80 residues long. The isolated domain is highly helical (>90% helix at 10 °C), forms a stable SAH in solution by CD and is monomeric. NMR confirmed that it forms a helical elongated structure, and the data suggests that salt bridges formed between side chains of E-R and E-K pairs are highly dynamic, rather than fixed. It is likely that this dynamic behaviour is important for the stability of SAH domains.

P-62

A comparative kinetic study of non-canonical eukaryotic translation initiation with IRES structures by single molecule fluorescence microscopy

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Protein synthesis is a complex multi-step process coordinated by the ribosome. Dynamics of such a process opens new interesting questions. In particular the dynamics of non-canonical translation initiation using IRES (internal recruitment entry site) structures remains largely unknown. This process is used by many viruses, as HIV, in order to hijack their host's translation machinery.

We are studying IRES initiation kinetics by single molecule total reflexion fluorescence microscopy. As translation is asynchronous, single molecule techniques may indeed be relevant to access the kinetic information. We monitored the motion of a single mammalian ribosome along a reporter system composed of a mRNA with an IRES at its 5' end. We performed a comparative study using different types of IRES in order to characterize their interaction with the ribosome in-vitro. Our work highlighted the existence of two characteristic times to describe the translation kinetics on mRNA with an IRES: a first long time, which could correspond to an interaction time between the ribosome and the IRES and a short time corresponding to the canonical elongation cycles.

P-64

Performance of cell free protein synthesis on single ribosome level

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Cell-free protein synthesis (CFPS) systems were designed to produce proteins with a minimal set of purified components, thus offering the possibility to follow translation as well as protein folding. In order to characterize the performance of the ribosomes in such a system, it is crucial to separately quantify the fraction of active ribosomes and the number of synthesizing cycles. We provide a direct and highly reliable measure of ribosomal activity in any given CFPS system, introducing an enhanced-arrest peptide variant. We observe an almost complete stalling of ribosomes that produce GFPem, as determined by common centrifugation techniques and FCS. Moreover, we thoroughly study the effect of different ribosomal modifications independently on activity and number of synthesizing cycles. Finally, employing two-color coincidence detection and two-color co-localization microscopy, we demonstrate real-time access to key productivity parameters with minimal sample consumption on a single ribosome level [1]. In a subsequent application we employed individually labelled 30S and 50S subunits which enabled us to study a potential dissociation of 70S ribosomes upon translation initiation [2].

[1] N. Kempf et al., (2017), *Scientific Reports*, in press

[2] C. Remes et al., (2017), submitted

Posters

– 2. Molecular machinery –

P-65

Single-molecule analysis of bovine mitochondrial F₁-ATPase

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F₁-ATPase (F₁) is one of the best-characterized molecular motors. The rotation dynamics of F₁ have been investigated in detail by extensive single-molecule studies and the structure of F₁ has been also determined under many conditions. However, the standard model F₁'s for single-molecule studies and structural analysis have been different; F₁ from thermophilic bacteria for single-molecule studies while bovine mitochondrial F₁ (bMF₁) for structural analysis. This prevents the conclusive determination of the correlation between crystal structures and rotational pausing states revealed in the single-molecule studies. In order to directly assign the crystal structures to rotational pausing states, we conducted single-molecule rotation assay of bMF₁. The rotation of bMF₁ was visualized by attaching an optical probe to the γ-subunit at a time resolution of up to 20 μs. We found two pause positions during 120 degrees rotation at saturating ATPγS concentration, and the time constants of each pause were determined to be ~9 ms and ~2 ms. The observation indicates that two or more elementary reactions of ATP hydrolysis after substrate binding occur at different angular positions, one of which is probably the ATP cleavage waiting state. We are now assigning these pauses to crystal structures.

P-67

Dynamics of the Bacterial Flagellar Motor

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The bacterial flagellar motor, a natural rotary machine, is driven by the ion flux cross the membrane with the interaction between stator and rotor. With the development of back-focal-plane detection technique, we are capable to observe the motor rotation at various loads. Many of the bacterial flagellar motor components turnover dynamically while motor rotation. To reveal the energetical dynamics of the stator-units, we perform single-motor speed tracking and motor speed population measurements. We use the strain with chimeric stator-units driven by Na⁺ ions. With controlling the number of the stator induction level and sodium-motive-force, we can further understand the energetic dynamics of the stators.

P-66

RecBCD enzyme repair dynamics in DNA double-strand breaks

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DNA Double-Strand Break (DSB) repair is essential for cell survival. In *E. coli* double-strand break repair is mediated by the RecBCD enzyme complex. The RecBCD complex locates the break and initiates the repair by homologous recombination.

Understand RecBCD dynamics is crucial to reveal how the DNA double-strand breaks are located, recognized and processed.

Currently, very little is known of the dynamics of DSB recognition and repair by RecBCD *in vivo*. Moreover RecBCD is expressed at very low levels, requiring study of this process to be conducted at the single molecule level.

We developed a fast imaging single molecule assay to track each subunit of RecBCD in real time. To observe single RecBCD molecules we label a single subunit of the complex with HaloTag-TMR and visualise with HILO (highly inclined and laminated optical sheet) microscopy.

Our single trajectory analysis allows us to investigate the dynamics of RecBCD during the repair process by measuring the apparent diffusion coefficient of RecBCD, and the average time it takes to reach its target.

P-68

Reconstruct energy landscapes and master equations from single-molecule traces a histogram approach

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Energy processing in biological systems is carried out by macromolecules called biomolecular motors. Motor dynamics is usually studied as a Brownian motion on a free-energy landscape. These landscapes consist of local energy barriers that separate metastable states. The motor will perform work as it undertakes random motion across these barriers creating a stochastic trace. Stochastic traces for a variety of molecular motors have been observed, these traces reveal important motor properties such as the velocity under load as well as the dwell times in observed metastable states.

Here we present a method for analysing the stochastic trace to reconstruct the landscape associated with the motor and determine whether a discrete master equation is a valid description of the system. The method uses the k-Space F-P equation and the trace histogram for this purpose. The reconstruct method was successfully used on a variety of motor regimes, while the criteria established for the applicability of master equation formalism agrees with previous results, suggesting that the preliminary results obtained are reliable.

Posters

– 2. Molecular machinery –

P-69

Pausing kinetics dominates strand-displacement polymerization by Reverse TranscriptaseO. Malik, H. Khamis, S. Rudnizky, A. Kaplan
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Reverse Transcriptase (RT) catalyzes the conversion of the viral RNA into an integration-competent double-stranded DNA, with a variety of enzymatic activities that include the ability to displace the non-template strand concomitantly with polymerization. Here, using optical tweezers to follow the activity of the Murine Leukemia Virus RT, we show that strand-displacement polymerization is interrupted by abundant pausing events. These pauses are modulated by the sequence ~8 bp ahead, indicating the existence of uncharacterized RT-DNA interactions. Most of the pausing events correspond to backtracking of the enzyme, whose recovery is also modulated by the DNA duplex. Finally, we show that re-initiation events, which induce long pauses and are likely the rate-limiting step in the synthesis of the genome *in vivo*, are modulated by the viral protein Nucleocapsid. Our results emphasize the potential regulatory role of conserved structural motifs, and may provide useful information for the development of potent and specific inhibitors.

P-71

Testing COI marker for molecular identification of caridean shrimp larvaeP. G. Morgante, T. M. N. Guimarães, D. P. Bezerra, G. Bertini
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Molecular identification is a powerful tool to determine the correct taxon of an individual, mainly in those cases whereas the morphological identification fails. Many DNA markers are available, but their efficiency to precise assign the species can vary depending on the animal group. The morphological identification of caridean shrimp larvae at the species level is a difficult task. There are a great number of species and the little larvae can be very similar to each other, remaining critical doubts to assign their species. Aiming to identify larvae collected from Rio Ribeira de Iguape, Vale do Ribeira/Brazil, we performed individual DNA isolation, PCR amplification of *COI* gene, using primers COIA and COIF, and DNA sequencing of PCR products. From the first thirty PCR products sequenced, only three resulted in good sequences. The BLAST (GenBank) analyses of these sequences suggested the presence of *Macrobrachium carcinus*, *M. olfersii* and *M. acanthurus* larvae species. The problems observed in the sequencing procedure suggested the presence of a heteroplasmic sample of PCR products obtained with primers COIA and COIF. Now, we are testing the efficiency of new primers for *COI* marker, LCO1490 and HCO2198, to allow the molecular identification of caridean larvae. Support: FAPESP/BR

P-70

Overstretching DNA and RNA double helices by optical trappingL. Melkonyan, M. Bercy, T. Bizebard, U. Bockelmann
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DNA undergoes a structural transition at forces above 50 pN, where the double helix elongates to about 1.7 times its crystallographic length. Recent experiments showed that strand peeling, bubble formation and cooperative unwinding might lead to DNA overstretching.

Here we report the first study of the overstretching of duplexes composed of DNA and RNA. Measurements are performed using a dual optical trap. One strand of the duplex is attached at both extremities to the two beads, while the other strand is attached only at one side. Therefore, the latter strand can peel under force and there is a single peeling front moving through the sequence in a predetermined direction. We experimentally studied the four possible cases, (i) DNA duplex, (ii) RNA duplex, (iii) DNA/RNA hybrid with DNA strand under tension, (iv) DNA/RNA hybrid with RNA under tension. The data are compared to a theoretical description of force-induced peeling and bubble formation.

We experimentally observe significant differences between the four cases. Duplexes containing at least one RNA strand overstretch at smaller forces than the DNA double helix. Most strikingly, we find that two different mechanisms cause the overstretching of the RNA double helix and the DNA double helix.

P-72

Macromolecular crowding modulates transport by teams of kinesin-1 motorsG. Nettesheim³, G. Jaffe³, S. J. King², G. T. Shubeita^{1,3}¹New York University Abu Dhabi, UAE; ²University of Central Florida, USA; ³The University of Texas at Austin, USA

The cytosol is crowded by a high concentration of macromolecules. Crowding can alter protein conformation, binding rates, and reaction kinetics, yet it is not known how crowding affects intracellular cargo transport by molecular motor proteins. We report on the consequences of crowding on cargo transport by kinesin-1 motors which we reconstituted *in vitro*. Surprisingly, we find that crowding significantly slows transport by teams of motors, while having no effect on single motor velocity. By applying controlled forces on single motors using the optical trap we find that this emergent property of kinesin teams results from the individual's increased sensitivity to hindering load in a crowded medium. This change in the motor response makes predictions on how cargoes transported by motor ensembles should respond to force, which we confirm using the optical trap. Based on the data, we propose a model by which entropic forces in the crowded medium lead to the motor's altered detachment characteristics, and suggest that crowding can be used as an additional control parameter in the quest for a better understanding of kinesin's mechanochemical cycle.

Posters

– 2. Molecular machinery –

P-73

Direct observation of dynamics in Type IV pili system and archaella motor

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Our group has developed techniques of optical microscopes that enable to visualize the molecular function of single motor proteins. We applied them to two microorganism subjects.

1. Type IV pili (T4P) system is a super-molecular machinery observed in prokaryote. Cells repeat the cycle of T4P extension, attachment to surfaces and retraction to drive twitching motility. Here we provided the framework of the T4P dynamics at single cell level in *Synechocystis* sp. PCC6803, which has the ability to recognize light direction. We demonstrated that the dynamics was detected by fluorescent beads under an optical microscope, and controlled by blue light that induces negative phototaxis; extension and retraction of T4P was activated at the forward side of lateral illumination to move away from the light source.

2. Motile archaea swim using a rotary filament, the archaelum, a surface appendage that resembles bacterial flagellum structurally, but is homologous to bacterial T4 pilus. Little is known about the mechanism by which archaella produce motility. To probe the mechanism, we applied microscope techniques to study a model organism, swimming *Halobacterium salinarum*, and computationally reconstructed the forward movement with structural and kinetic parameters obtained by measurements.

P-75

Conformational transition from catalytic dwell to ATP-binding dwell in F₁-ATPase

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F₁-ATPase (F₁) is the catalytic part of ATP synthase that synthesizes most of ATP in living organisms. Extensive crystallographic and single-molecule studies have clarified atomic structures and mechanochemical coupling of this unique rotary motor, respectively. However, connection between these two aspects are not well established: structural basis of the functional cycle has been controversial. Here, we use molecular dynamics simulation to clarify conformational cycle of the molecular motor, integrating information from recent crystallographic and single-molecule studies. Particularly, we resolve conformational transition from the catalytic dwell to the ATP-binding dwell that involves a 40° rotation of the rotor.

P-74

Stator mechanosensitivity of the bacterial flagellar motor probed by load manipulation

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The bacterial flagellar motor (BFM) is the multi-component complex which powers the swimming and swarming of many motile bacteria. The torque of this rotary motor is provided by stators, ion motive force powered ion channels which are known to assemble and disassemble dynamically in the BFM. Recently, it has been observed that this turn-over is mechano-sensitive, with the number of engaged stators dependent upon the external load acting on the motor. Despite their central role in the function of the BFM, a systematic study of the stator dynamics is lacking. Here we provide a quantitative and non-invasive measurement of the temporal behavior of the stators active in the BFM of *E. coli*, by estimating stator stoichiometry from high-resolution single-motor torque traces, quantifying for the first time the dependence between stator number and external load at steady-state. Furthermore, a rapid and controlled change in the external load, applied via a magnetic field, allows us to directly probe BFM mechano-sensitivity, systematically triggering and detecting stator association and dissociation. We incorporate these results into an adsorption model of stator kinetics, providing the first step into understanding the mechanism of mechano-sensitivity of the BFM.

P-76

Proofreading of DNA polymerase: a new kinetic model with higher-order terminal effects

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High fidelity of DNA replication is crucial for cell. The replication process is catalyzed by DNA polymerase (DNAP) which usually contains a polymerase(P) site and an exonuclease (E) site. Both sites contribute to the overall replication fidelity, but how they cooperate is not yet quantitatively understood. There were quite few theoretical studies on this issue. Recently we proposed a comprehensive kinetic model which includes previous models as special cases[1]. The basic idea is that there can be nearest or even higher-order neighbor interactions between the incoming nucleotide and the terminus of the nascent chain. By using the general method to handle copolymerization problems established previously by us [2], we obtained analytical solutions of the corresponding kinetic equations to address the fidelity issue systematically. We showed that the higher-order terminal effects can contribute substantially to the fidelity and the polymerization-proofreading mechanism is dominated only by very few key parameters. We applied the results to calculate the fidelity of some real DNAPs, which are in good agreements with previous intuitive estimates given by experimentalists.

[1] J. Phys.: Condens. Matter 29(2017)025101

[2] J. Phys.: Condens. Matter 27(2015)235105.

Posters

– 2. Molecular machinery –

P-77

Metabolically-tuned levels of SBF and MBF are key factors controlling Start in budding yeastS. Tollis², S. Dorsey¹, M. Cook³, J. Cheng², L. Black¹, C. A. Royer¹, M. D. Tyers²¹Dept. of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY, USA; ²Institut de Recherche en Immunologie et Cancérologie, Univ. de Montréal, Canada; ³Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

How cell size control is achieved remains a fundamental unsolved problem, largely due to a lack of reliable quantitative information. Start, the commitment to division in *S. cerevisiae*, is associated with a MBF (Mbp1/Swi6) and SBF (Swi4/Swi6) controlled transcription burst. We have used scanning Number and Brightness microscopy to reliably measure Mbp1, Swi4/6 and Whi5 (SBF repressor) protein levels in single live yeast cells. We find that these factors exhibit low nuclear concentrations (70–200nM) and form dimers. While Swi4 and Mbp1 compete for Swi6, SBF/MBF do not saturate target DNA sites. Strikingly, [Swi4] increases with cell size in G1. Mathematical modeling positions the [Swi4]/[Whi5] ratio and the fraction of SBF/MBF-bound DNA sites as new key determinants of Start. Moreover, we find that MBF components are upregulated (+50%) in cell grown in glycerol. In agreement with our model predictions, unlike wild-type the *mbp1* deletion mutant is only slightly smaller on poor compared to rich medium, reflecting a leading role for MBF in driving premature Start and small cell size on poor nutrients. These results reveal that metabolically-driven changes in Start factors expression ratios exercise significant control over the balance between growth and division in budding yeast.

P-79

Possible scenarios of DNA unzipping process

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DNA unzipping plays a key role in the gene information transfer processes in living cells. Data obtained from single-molecule micromanipulation experiments shows some unexpected features. On the force-distance curve for λ -phage DNA two consequent plateaus with the difference of the order of 2 pN are observed. DNA unzipping is followed by peaks occurrence in the plateau area with amplitude that decreases to the end of the process.

To understand unzipping process we calculate the opening energies for A·T and G·C base pairs along different pathways using atom-atomic potential function method. It is determined that DNA unzipping process can take place according to two scenarios. In the first scenario base pairs firstly transit into the 'pre-opened' state along the pathway 'opening' and then fully open along the pathway 'stretch'. In the second scenario base pairs open directly along the pathway 'stretch'. It is shown that in the first scenario due to bistability occurrence between the 'pre-opened' and 'opened' states, unzipping process flows cooperatively. In the second scenario due to DNA heterogeneity the non equilibrium opening process takes place. The first scenario is more appropriate for the real processes in living cell.

P-78

Interaction of cisplatin with DNA and Proteins Studied by FTIR Spectroscopy and SAXSV. Travkina¹, I. Belaya¹, M. Arefiev¹, E. Chikhirzhina², A. Polyanichko^{1,2}¹Saint-Petersburg State University, Russian Federation;²Institute of Cytology of the RAS, Russian Federation

Diamminedichloroplatinum(II) (DDP) is one of the most known and widespread anticancer drugs. The biological activity of the compound is arises from its ability to interact with DNA and proteins. However, molecular mechanisms of these interactions are not clear yet.

We studied complexes of serum albumins with *cis*- and *trans*-isomers of DDP using FTIR spectroscopy and Small Angle X-ray Scattering (SAXS). We have shown that both of the DDP isomers facilitate the formation of BSA dimers. The observed effect is stronger for the *trans*-DDP. Based on the data obtained we conclude that the observed intermolecular binding are due to the changes in the BSA secondary structure, induced by DDP binding. Also, we investigated the interaction of DNA with the DDP by FTIR-spectroscopy. The applicability of the developed approach to the analysis of FTIR spectra of DNA complexes is discussed.

Part of the study was carried out in the Center for Optical and Laser Materials Research and Center for Diagnostics of Functional Materials for Medicine, Pharmacology and Nanoelectronics of SPbU and National Research Center «Kurchatov Institute», the authors are grateful to G. Peters for help in SAXS measurements. The work was supported by Russian Foundation for Basic Research (grants 15-04-06993, 15-08-06876).

P-80

Mitochondrial dysfunction in neurodegenerative diseases associated with RNA binding proteinsL. Zhu¹, J. Deng¹, P. Wang¹, J. Liu¹, J. Y. Wu^{1,2}¹State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; ²Department of Neurology, Center for Genetic Medicine, Lurie Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, USA

Many mutations have been identified in the human genes encoding nuclear RNA binding proteins (RBPs), such as TDP-43 and FUS, among patients affected by amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), two groups of heterogeneous neurodegenerative diseases. Both TDP-43 and FUS shuttle between the nucleus and cytoplasm. A number of studies including ours suggest that the nucleus-cytoplasm shuttling and excessive cytoplasmic distribution of these RBPs contribute to neurotoxicity and disease pathogenesis. Using biochemical, molecular and cell biological assays together with analyses of cellular and animal models as well as patient samples, we have demonstrated that TDP-43 protein is localized to mitochondria. Increased expression of wild type or ALS-mutant proteins elicits mitochondrial impairment and subsequent neuronal death. Mutant peptide of TDP-43 adopts an anti-parallel β -structure analyzed by NMR study. Our genetic analyses reveal a couple of genetic modifier genes for TDP-43 associated FTLD and ALS. The results provide a mechanistic explanation for pathogenesis and suggest that mitochondrial dysfunction may be a target in future development of diagnostic and therapeutic tools for TDP-43 proteinopathy.

Posters**– 3. Quantitative approaches to gene regulation –****P-81 (O-25)****Heat triggers specific mRNA localization to regulatory RNA-protein granules in budding yeast**E. W. J. Wallace¹, C. D. Katanski², D. A. Drummond²¹University of Edinburgh, UK; ²University of Chicago, USA

Eukaryotic cells form large RNA-protein granules in response to stress. Here we measure the RNA content of heat-shock granules or aggregates by sequencing RNA from distinct fractions of cell lysate. We find that most messenger RNA species (mRNAs) aggregate specifically in response to heat shock. This is consistent with our reported measurements of heat-aggregated proteins, which were enriched in RNA-binding proteins. However, many specific mRNAs are excluded, including many messages that are abundant prior to heat shock, and also newly transcribed heat-shock protein mRNAs. Proteins are synthesized from only a subset of non-aggregating RNAs. No mRNAs remain aggregated during recovery at normal growth temperature, consistent with observed reversible aggregation of protein components of heat shock granules. RNA-binding proteins and their measured mRNA binding partners may aggregate together, or independently, suggesting stress-triggered unbinding. Our results are consistent with heat-shock granules being sites of temporary translational repression for specific mRNAs, acting in parallel with transcription, translation, and degradation of RNA to remodel cells in response to stress.

P-82 (O-24)**Nucleosome mobility and the regulation of gene expression: insights from single-molecule studies**

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Nucleosomes at the promoters of genes regulate the accessibility of the transcription machinery to DNA, and function as a basic layer in the complex regulation of gene expression. Our understanding of the role of the nucleosome's spontaneous, thermally driven position changes in modulating expression is lacking. This is the result of the paucity of experimental data on these dynamics, at high-resolution, and for DNA sequences that belong to real, transcribed genes. We have developed an assay that uses partial, reversible unzipping of nucleosomes with optical tweezers to repeatedly probe a nucleosome's position over time. Using the nucleosomes at the promoters of two model genes, *Cga* and *Lhb*, we show that the mobility of nucleosomes is modulated by the sequence of DNA and by the use of alternative histone variants, and describe how the mobility can affect transcription, at the initiation and elongation phases.

P-83**Functional phenotypic variability via growth rate mediated feedback in *Escherichia coli***O. Patange^{1,2}, C. Schwall^{1,2}, D. Griffith¹, A. Phillips³, J. Locke^{1,2}¹The Sainsbury Laboratory, University of Cambridge, Cambridge, UK; ²Department of Biochemistry, University of Cambridge, Cambridge, UK; ³Computational Science Laboratory, Microsoft Research, Cambridge, UK

Gene expression of key regulators is often noisy and dynamic in individual cells. The cellular environment and growth can modulate the observed single cell dynamics, but it remains unclear how or why. In this study we use *rpoS*, the master stress response regulator in *E. coli*, to address how cells couple growth to gene expression to generate functional phenotypic variability. We find *rpoS* is highly expressed in a small fraction of cells of an unstressed population. This small fraction survives when challenged by oxidative stress while most other cells die. Using single-cell, time-lapse microscopy and microfluidics we demonstrate that multi-generation *rpoS* pulses stochastically produce this sub-population. An initial slowdown in growth rate allows cells to generate these pulses via a dynamic feedback mechanism. We develop a stochastic model of the growth and *rpoS* feedback. The model predicts that decreasing the average population growth rate will increase the fraction of cells with high *rpoS*, for which we find experimental agreement. Our work illustrates the importance of the coupling between cell physiology and global gene regulation in producing functional phenotypes.

Posters

– 4. Synthetic biology –

P-84

Nanoparticle-based electrochemical sensor for antioxidant activity monitoring in plant extractsM. Florescu¹, M. David^{1,2}, A. Serban¹, C. Radulescu³, C. Stih³, D. Lazurca⁴¹Transilvania University of Brasov, Brasov, Romania; ²University of Bucharest, Măgurele, Romania; ³Valahia University of Targoviste, Romania; ⁴Nutraceutical SRL, Sacele, Romania

Oxidative stress is important as it plays a major role in a variety of chronic and degenerative diseases. Various plants contain high concentrations of several antioxidants, which can inhibit the oxidation of biomolecules by the free radicals that are formed in living organisms. Our aim is to provide rapid access at a global level for total antioxidant activity of real samples. In this work hydrosoluble plant extracts with antioxidant activity were obtained via two physical extraction methods. Carbonic and gold nanoparticle modified screen-printed carbon electrodes have been successfully used to develop sensitive, label-free electrochemical sensors for total antioxidant activity monitoring. Optimization studies by means of pH, working potentials, type of nanoparticles and extracts were carried out. The results were validated and correlated using the classical methods of spectroscopy (UV-VIS, FTIR and Raman). Highest antioxidant activity and optimized sensors configuration have been outlined. These results determine the sensors applicability in real samples such as plant extracts and in the future for blood serum.

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P-86 (O-29)

Artificial cell reactor array technologyH. Noji^{1,2}¹ImPACT Artificial Cell reactor program, JST, Japan; ²Applied Chemistry, The University of Tokyo, Japan

We developed two classes of array devices displaying over one million of femto-liter reactors (FR); water-in-oil droplet type and lipid bilayer-sealed type. Both of them have size in a few microns and volume of femtoliters. We have demonstrated digital bioassays with droplet FR; digital ELISA, and digital virus counting. Droplet FR also allows us the integration of in vitro gene expression system from single DNA molecules in femtoliter space. A surprising feature of this system is the small variance of gene expression yield, 15% that is remarkably smaller than that of in-cell expression. Taking this advantage, we successfully conducted directed evolutions of enzymes. Arrayed lipid bilayer chamber (ALBiC) displayed the femtoliter reactors, each sealed with a lipid bilayer sheet on top. Single-molecule analysis of membrane transporters was first conducted. Then, to explore the feasibility to form a self-replicating molecular system on the device, we generated a hybrid system of bacterial cell and ALBiC by fusing bacterial protoplast into lipid bilayer. Some of hybrid reactors showed active gene expression and also budding of vesicle structures although in only few cases. In the presentation, I will concisely introduce the latest state of artificial cell reactor project.

P-85

DNA origami dimensions and structure measured by solution X-ray scatteringM. A. Baker¹, A. J. Tuckwell¹, J. F. Berengut¹, J. Bath², F. Benn², A. P. Duff³, A. E. Whitten³, K. E. Dunn⁴, R. M. Hynson⁵, A. J. Turberfield², L. K. Lee^{1,6}¹EMBL Australia, UNSW, Australia; ²University of Oxford, UK; ³ANSTO, Australia; ⁴University of York, UK; ⁵VCCRI, Australia; ⁶EMBL, Hamburg, Germany

The rational design of complementary DNA sequences can be used to create nanostructures that self-assemble with nanometre precision. These “DNA origami” are typically imaged by atomic force and electron microscopy. Small-angle X-ray scattering (SAXS) provides complementary structural information on the ensemble-averaged state of DNA origami in solution. Here we demonstrate that SAXS can distinguish between different single-layered DNA origami that look identical when imaged with AFM. We use SAXS to quantify the magnitude of global twist of DNA origami tiles with different crossover periodicities highlighting the extreme structural sensitivity of single-layered origami to the location of strand crossovers. We show that SAXS can quantify the distance between pairs of gold nanoparticles tethered to specific locations on a DNA origami tile and use this method to measure the overall dimensions and geometry of the DNA nanostructure in solution. Finally, we use indirect Fourier methods to measure the distance between DNA helix pairs in a DNA origami nanotube. Together, these results provide important methodological advances in the use of SAXS to probe the solution state of DNA origami.

P-87 (O-30)

Bioinspired sensor material for broad-banded molecular scale, wavelength selective detectionJ. Vinals, P. Judge, A. Watts
University of Oxford, UK**Abstract**

In the past 50 years, silicon devices such as transistors have been developed, miniaturised and increasingly integrated into numerous items used on a daily basis. Miniaturisation has become the critical step and it is predicted that the size limit of silicon will be reached within the next decade. It is thus necessary to find alternatives, and using functional biomolecules is currently one of the most promising options. Bacteriorhodopsin (bR) is a robust light-driven proton pump found in the purple membrane of the halophilic organism *Halobacterium salinarum*. A photoelectric response of bR was first observed over three decades ago, and the electrical conductance behaviour of the protein has been studied since then. Such studies, though, were only performed within the membrane.

Here, we plan to develop the wavelength tuning potential of bR in electronic devices, either as a photodetector, or as an imaging device, with the molecular scale detector being ~5nm in size, based on our previously published work

Posters

– 4. Synthetic biology –

P-88 (O-31)

The development of hybrid biomaterials for regenerative engineering

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Reengineering cells to operate effectively in biological systems invariably involves the assembly of multiple components, which can only be integrated when compatible interfaces are built into the design. This can be achieved through the synthesis of hybrid materials comprising highly cooperative biological and synthetic constituents that can be used to amplify or attenuate host tissue interactions. The systems methodology that underpins this design approach provides a gateway to the development of non-traditional approaches to regenerative medicine. Accordingly, I describe three emerging research programmes that span the fields of synthetic biology, biomaterials, and regenerative engineering: *Stem Cell Painting*¹, where artificial membrane binding proteins undergo spontaneous assembly at the plasma membrane of stem cells; *Cell Paintballing*², where microdroplet vectors are controlled optically to deliver bimolecular payloads to specific membranous regions of individual cells; and *Three-Dimensional Bioprinting*³, where a new hybrid microporous bioink is used to print stem cell laden tissue-like constructs.

1. Armstrong et al., *Nature Communications* **2015**, 6, 7405.
2. Armstrong et al., *Chemical Science* **2015**, 6, 6106.
3. Armstrong et al., *Advanced Healthcare Materials* **2016**, 5, 1724.

P-90

Determining the roles of Caf1R gene products in the expression of Caf1 polymers by recombinant *Escherichia coli*

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As a form of virulence factor, *Yersinia pestis* the causative agent of plague produces a polymeric protein Caf1 (F1 capsule) at 37° C in order to resist the phagocytosis by host's macrophages. The expression of polymeric Caf1 is accomplished by the cooperation of four different proteins which are encoded by four structural genes. The Caf1 subunit is the capsule building block whilst the Caf1M chaperone prevents Caf1 subunits from aggregation and protease digestion in the periplasm and transports them to the usher protein in the outer membrane. This Caf1A usher plays a crucial role in the polymerization of Caf1 subunits and secretion of the growing polymers from the bacterial cell surface. The Caf1R protein is a potentially temperature-sensitive transcriptional regulator of capsule expression. Our aim is to understand Caf1R regulation in order to optimize the production of Caf1 polymers for synthetic biology and biotechnology applications. So far our results show that Caf1R is required for Caf1 production (positive regulator) but over expression of Caf1R is inhibitory. On the other hand when Caf1R was deleted in the presence of a T7 artificial promoter, the production of Caf1 was highly increased. In one example, the yield of Caf1 in the culture supernatant was enhanced by more than 50 % after deletion of Caf1R.

P-89

G-quadruplex: G-rich DNA sequences like potassium biosensorL. Bruni^{1,3}, M. Manghi^{1,2}, S. Croci^{1,2}

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The goal of this work is to study, with CD spectroscopy, a K⁺ biosensor in water solutions, done by DNA single strand sequences Pu23 and Pu27. These guanine rich strands, that show G-quadruplex (G4) folding, are formed by two or more tetrads stacked on each other. The tetrad is an array formed by four guanines paired by Hoogsteen bonds. Both stable stacking and folding structure are due to: hydrophobic interactions amongst the aromatic rings of guanines; H bonds amongst the guanines; Na⁺ and K⁺ role. The pivotal function of cations is exploited by coordination of the carbonyl oxygens of guanines, leading to G4 stabilization. K⁺ shows a higher affinity than Na⁺, because K⁺ coordinates eight oxygens and Na⁺ four. Pu27 and Pu23 are investigated as K⁺ biosensor in complex water solutions. DMEM, a water solution with Na⁺ and K⁺ ions, is added with K:D-rib (water solution of D-ribose and KHCO₃) 5mM. Several dilutions of DMEM are tested as ion solutions promoting G4 folding. To better exploit the Na⁺ and K⁺ roles on sensor activity, Pu27 and Pu23 folded at the lowest concentration, are titrated with NaCl and KCl solutions. CD spectra of Pu27 and Pu23 folding at the Na⁺ and K⁺ concentrations obtained by titration are presented.

P-91

Use of fluorescence spectroscopy in synthetic biologyM. Hof¹, M. Amaro¹, J. Sykora¹, J. Brezovsky², Z. Prokop², R. Chaloupkova², S. Kovacova³, V. Nemec³, K. Paruch³

¹J. Heyrovský Inst. of Physical Chemistry; CAS; Prague, Czech Republic; ²Loschmidt Labs, Dep. of Experimental Biology, Masaryk Uni, Brno, Czech Republic; ³Dep. of Chemistry, Faculty of Science, Masaryk Uni, Brno, Czech Republic

Enzymes' substrate specificity is linked to the active site properties. We report two fluorescence methods that specifically senses hydration and mobility in the active site vicinity of dehalogenase enzymes: A) "Time-dependent fluorescence shifts"^{1,2} (TDFS) of dyes, which are made part of a substrate. B) Decomposition of steady-state spectra of a fluorescent unnatural amino acid³; site-specifically incorporated into the protein. We use this approaches to understand why natural enzymes have superior selectivity than enzymes created by De novo protein design. We address this question by transplanting the active site from a dehalogenase enzyme with high enantioselectivity (ES) to a nonselective dehalogenase enzyme. Crystallography confirms that the active site geometry of the redesigned dehalogenase matches that of the target, but its ES remains low. TDFS and simulations reveal that dynamics and hydration at the tunnel mouth differ substantially between the redesigned and target dehalogenase. Our results emphasize the importance of dynamics and hydration for rational protein design⁴. 1 Jesenska, et al. *JACS* **2009** 494. 2 Amaro, et al. *J Phys Chem B* **2013** 7898. 3 Amaro, et al. *JACS* **2015** 4988. 4 Sykora, et al. *Nature Chem Biol* **2014** 428

Posters

– 4. Synthetic biology –

P-92

Characterization of new calpain inhibitors based on an intrinsically disordered protein, calpastatinH. H. Nguyen^{1,2}, M. Varadi^{1,4}, K. Pauwels^{1,2}, P. Tompa^{1,3}¹VIB Center for Structural Biology, Vlaams Instituut voor Biotechnologie, Brussels, Belgium; ²Structural Biology Brussels, Vrije Universiteit Brussel, Brussels, Belgium; ³Institute of Enzymology, Research Centre for Natural Sciences of the Hungarian Academy of Sciences, Budapest, Hungary; ⁴European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridge, UK

Intrinsically disordered proteins (IDPs) are a new important class of proteins, which involve in many biological processes and human diseases. Despite the increasing number of newly discovered IDPs, these proteins have not yet been considered in the field of protein design. In this project, novel IDPs that were designed using computational approaches will be produced and characterized in vitro to validate their designed functionality through binding to a target protein. Human m-calpain has been chosen as the first target of the project because: (1) its involvement in many biological processes and diseases; (2) its endogenous inhibitor, calpastatin, is an IDP; (3) detailed structural information of the interaction between calpain and calpastatin is readily available. In our study, a set of peptides were designed in silico for the individual binding sites on the surface of calpain and based on the interaction of those peptides to calpain, good binders will be selected to generate a small library of IDPs that should bind to and inhibit calpain. At the end of this project, we intend to prove that the designed IDP has its expected characteristics and functionality in vitro and potentially provide a reliable method to design IDPs.

P-94

Toward reproduction of a bacterium from hybrid chamber cellsK. V. Tabata^{1,2}, Y. Moriizumi¹, R. Watanabe¹, H. Noji^{1,2}¹The University of Tokyo, Japan; ²ImPACT, Cabinet Office, Japan

The Gram negative bacteria *E. coli* has a lipid bilayer made of peptidoglycan. It is known that spheroplasts and protoplasts can survive to some extent the loss of the outer membrane, but perish if the inner membrane is also compromised. From this observation, one could propose that maintaining distinct inner and outer membranes is key to survival. To test this theory, we manipulated the membrane structure. We prepared a microchamber that had the same volume as an *E. coli* cell and also a lipid membrane. By fusing the chamber with an *E. coli* cell, we could introduce into the chamber *E. coli* cytoplasmic material and into the lipid membrane of the chamber *E. coli* membrane proteins. Doing so, we could envelope the fused material with an artificial lipid membrane. We confirmed the cell retained metabolic ability and replicative ability. Prior to the fusion, we had inserted a plasmid into the chamber. Following the fusion, we observed plasmid protein synthesis. Additionally, although rare, we also observed living *E. coli*-like organisms from the fused cells. These results suggest inanimate conditions from which life can form.

P-93

Computational investigation and design of calcitonin-based amyloidsF. Rigoldi¹, A. Redaelli¹, P. Metrangola², A. Gautieri¹¹Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy; ²Dipartimento di Chimica, Materiali e Ingegneria Chimica “Giulio Natta”, Politecnico di Milano, Via L. Mancinelli 7, 20131 Milano, Italy

Calcitonin is a 32 amino acid thyroid hormone that can form amyloid fibrils. The DFNKF peptide (corresponding to residues ranging from Asp15 to Phe19 of human Calcitonin) is the minimal amyloidogenic unit. We investigate fibrils formed by the DFNKF peptide by simulating different arrangements of this amyloidogenic core sequence. We show that DFNKF fibrils are highly stable when assembled in parallel β -sheets, while they quickly unfold in antiparallel conformation.

The insights into DFNKF amyloids can be crucial for nanobiotechnological application, thanks to the possibility to tune their aggregating properties by modify the peptide structure. Here we introduce different chemical modifications in different positions and computationally test the thermal stability of the amyloids and we provide insights into the newly introduced stabilizing interactions. The experimental test of the modified peptides show an increased stability of DFNKF gels, which is highly dependent on the type and position of chemical modification.

P-95

DNA T-junctions for studies of DNA origami assembly

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DNA T-junctions, as first reported by Hamada & Murata in 2009[1], are an alternative to cohesive sticky-ends for the joining of two DNA duplexes within a nanostructure. Hybridisation occurs between a single-stranded loop on one of the duplexes and a complementary sticky-end on the second duplex, forming a rigid T-shaped motif. Here we present the results of a study of the physical characteristics of these junctions, including the effects of loop length and sequence on T-junction geometry and stability. These analyses include T-junctions assembled with a 3' sticky end, which to our knowledge have not yet been used to construct DNA nanostructures. The results of coarse-grain modelling simulations of the junctions using OxDNA[2] have been used to assist in the interpretation of experimental results and to guide experimental design. Finally, we present the design of a ‘DNA origami-like’ tile which utilises T-junctions. The tile design allows the number and layout of uniquely-addressable domains used by linking ‘staples’ to be programmed by the addition or omission of only a small number of DNA strands, facilitating studies of origami assembly.

References:

- [1] Hamada, S. & Murata, S. *Angew. Chemie - Int. Ed.* **48**, 6820 (2009)
- [2] P. Šulc *et al.*, *J. Chem. Phys.* **137**, 135101 (2012)

Posters

– 5. Protein-nucleic acid interactions –

P-96 (O-35)

Real-time investigation of the assembly dynamics of artificial virus-like particlesM. Marchetti¹, D. Kamsma¹, R. de Vries², W. H. Roos³, G. J. L. Wuite¹¹Department of Physics and Astronomy and LaserLaB Amsterdam, Vrije Universiteit Amsterdam, Netherlands;²Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Netherlands; ³Moleculaire Biofysica Zernike Instituut, Rijksuniversiteit Groningen, Netherlands

Artificial viruses are model systems for the understanding of natural viruses and potential vehicles for genetic material delivery. It is still a challenge to fully reproduce the natural viral cooperativity behavior during the self-assembly process[1], therefore we are working with simplified model systems in which we can easily and freely tune interaction parameters. This study focuses on the assembly kinetics of an artificial polypeptide designed to self-assemble into a rod-shaped virus-like particle on double stranded DNA[2]. We employed optical tweezers which allowed us to suspend the DNA tether in solution and to monitor by confocal fluorescence microscopy the peptide binding, unbinding and sliding along the DNA in real-time using fluorescently labelled peptides[3]. We also employed acoustic force spectroscopy (AFS) as complementary technique for monitoring the real-time self-assembly at low forces (1–2 pN)[4]. This work opens the doors for new insights into the assembly process of rod-shaped artificial and natural viruses.

[1] *Nature Physics* 2010,6,733–743, [2] *Nature Nanotech.* 2014, 9,698–702, [3] *Methods in Enzymology* 2017,582,85–119, [4] *Nature Methods* 2015,12,47–50

P-98 (O-37)

Structural information of PTBP1/EMCV complex by combining orthogonal spin labelling with pulse EPRC. Gmeiner¹, G. Dorn², F. Allain², M. Yulikov¹, G. Jeschke¹¹ETH Zurich, Institute of Physical Chemistry, Switzerland;²ETH Zurich, Institute of Molecular Biology & Biophysics, Switzerland

The combination of site-directed spin labeling (SDSL) with pulse Electron Paramagnetic Resonance (EPR) spectroscopy, such as 4-pulse Double Electron-Electron Resonance (DEER), emerged as a promising tool in structural biology to obtain information on site-to-site distances in a range of 1.5 to 8.0 nanometer. Besides the well-established nitroxide radicals, high-spin systems such as Gd(III) based complexes were studied extensively. Combinations of these spin labels turned out to be a powerful technique to get more distance information and thus it offers a valuable method for unknown structures.

Here, we present a calibration method for different spin labels by measuring protein-protein distance distributions in individual RNA binding domains of the alternative splicing regulator Polypyrimidine-Tract Binding Protein 1 (PTBP1). For our system, the complex of PTBP1 and the Encephalomyocarditis Virus (EMCV) RNA, we follow the approach of orthogonal SDSL to obtain structural information in terms of orientation and arrangement by attaching Gd(III)-based spin labels on the protein and nitroxide radicals on the RNA. We aim then to combine short-range NMR restraints with long-range EPR restraints to determine the 3-dimensional structure of this protein/RNA complex.

P-97 (O-36)

NMR reveals how phosphorylation of the retinoic acid nuclear receptor regulates gene expression

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Nuclear Retinoic Acid Receptors (RARs) are ligand-dependent transcriptional regulators, which mediate the effects of RA, the major active metabolite of vitamin A. RAR shares a common architecture with all other nuclear receptors that includes a ligand binding domain (LBD), a DNA binding domain (DBD) involved in the recognition of hormone specific response elements and an N-terminal intrinsically disordered region (NTD). Recent studies highlighted the importance of the topology of RARs DNA cognate sequences and kinase signalling pathways for fine spatio-temporal regulation of RAR-target genes expression. We have been studying by NMR the molecular mechanism by which the phosphorylation of a conserved serine within the intrinsically disordered region modulates the gene activation of RAR γ . This mechanism involves the phosphorylation-dependent modulation of the affinity between a proline-rich region within the NTD and a SH3 domain of the vinxin β , a RAR γ co-regulator. Our results highlight a complex allosteric mechanism linking RAR γ DNA binding properties with its phosphorylation state at the NTD disordered region. Belorusova AY, *Biochemistry* (2016) 55, 1741

Martinez-Zapien D, et al. (2014) *Protein Expr Purif*, 95C, 113Lalevée S, et al. (2010) *FASEB J*, 24, 4523

P-99

DNA-Protein interactions rule in DNA binding specificity of Androgen and Glucocorticoid receptors

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The nuclear receptors are transcriptional regulatory factors that control gene expression. The androgen and glucocorticoid receptors, members of this nuclear receptor family bind as a homodimer to identical genome response elements, which consist of two hexameric half-sites (5-AGAACA-3) arranged as inverted repeat with 3bp of separating DNA. However, GR fails to bind to direct repeats whereas AR forms stable enough protein-(DR)DNA complex to allow crystallization. This different behavior, which can be regarded as a crucial key in specific regulation upon hormone binding, has been questioned to originate by different aspects of interaction. Beside of the state of dimer formation of complexes, the others key factors, suggested by experimental studies are affinity rate constant [K^{off} , K^{on}] of each monomer to its respective DNA hexamer as well as nearby genes effect on complex activation. To address these mechanism-based questions, we used biased and unbiased MD simulation for both complexes in dimer/monomer states with their relative mutations as well as considering their flanking sites. Our result suggest that first monomer has higher affinity than second monomer to its relative hexameric response element and second monomer shows more dynamic regarding to mutated flanking sequences.

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– 5. Protein-nucleic acid interactions –

P-100

The mechanism of branch migration during DNA strand displacement

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DNA strand displacement is crucial to fundamental biological processes such as homologous recombination and is a vital tool in DNA nanotechnology. Toehold-mediated strand displacement begins as a single nucleic acid strand invades a partial duplex. The reaction is thought to proceed through branch migration with the two dangling strands competing to basepair with a third complementary strand until, ultimately, the longer strand displaces the shorter strand. Little is known about the mechanism and kinetics of branch migration as most studies are performed in bulk where bimolecular toehold formation dominates the kinetics. With single-molecule FRET, we experimentally determine the distribution of displacement times post toehold formation. We explore the effect of salt, temperature, length, and sequence on branch migration. Further, we demonstrate that waiting time distributions are very sensitive to the direction of invasion, and the effect reverses when the invader is composed of RNA. Finally, we rationalize this behavior through previous knowledge of dangling end stability to provide a more detailed microscopic description of branch migration.

P-102

DNA synthesis determines the binding mode of the human mitochondrial SSB proteinsF. Cerrón^{1,2}, J. A. Morín³, J. Jarillo², E. Beltrán², G. L. Ciesielki⁴, F. J. Cao², L. S. Kaguni⁴, B. Ibarra¹

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The human mitochondrial single-stranded DNA binding protein (HmtSSB) binds single-stranded DNA (ssDNA) with high affinity and defines the nucleoprotein substrate upon which DNA replication and repair processes must act. It is, therefore, indispensable for mitochondrial and cell survival. We used optical tweezers to measure the binding properties of the HmtSSB proteins to long ssDNA molecules and their elastic and energetic properties. Modeling of the force extension curves of individual nucleoprotein complexes revealed that the HmtSSB proteins use two binding modes to organize the ssDNA, which depend on the ionic strength and protein concentrations. Different binding modes may be related to different functions. We investigated if any of these binding modes is preferentially used when protein binding is coupled to DNA replication. One binding mode was selected for all experimental conditions. These results reveal the role of the gradual release of ssDNA during replication on regulating the HmtSSB binding mode and on generating the appropriate nucleoprotein structure for subsequent replication of the displaced strand. Similarly, the gradual release of ssDNA during other DNA metabolic processes is expected to regulate the binding modes of SSB proteins exposing multiple OB folds.

P-101

Mechanical impact of DNA bubbles on single molecules of thousands of base pairsA. Brunet¹, P. Rousseau², L. Salome³, N. Destainville⁴, M. Manghi⁴, C. Tardin³

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The DNA sequences that are less stable with temperature constitute starting points for replication and transcription. AT rich sites, which are known to melt at lower temperature than GC rich ones, are all found in regions flanking genes. It is now commonly thought that these regions undergo local denaturation in the physiologically relevant range of temperature and form so-called DNA bubbles. Indeed, during the last decade, several biophysical studies have shown an unexpected increase of the DNA flexibility as a function of the temperature, which could be accounted for by theoretical simulations with local denatured sequences, inserted in the DNA molecule. Here, we question the existence of bubbles in dsDNA under physiological conditions through their mechanical impact on DNA molecules ranging from several hundreds to thousands base pairs. We obtain data that strikingly differ with previous published results, which we discuss thoroughly.

P-103

Visualizing CTCF-mediated DNA looping at the single-molecule levelM. E. Fuentes Perez¹, K. Blozelyte², M. Merckenschlager^{1,2}, D. Rueda^{1,2}

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Long-distance interactions in chromatin, where distant DNA regions are brought together to spatially organize the genome, play important roles during replication and gene regulation. CTCF is an essential nuclear protein highly conserved from flies to humans that it is implicated in multiple roles, including chromatin division, gene activation and repression, and genome looping among others. CTCF contains 11 zinc fingers that can bind up to 35 bp of DNA including the well-conserved CCCTC sequence. Moreover, the binding sites orientation play an important role in the direction of looping formation. Here, we have used smFRET to monitor and characterize CTCF-mediated DNA looping. To this aim, we have designed fluorophore-labelled DNA substrates (Cy3 and Cy5) with specific CTCF binding sites, such that loop formation results in a FRET signal. Our data show that loop formation is sequence specific and increases with protein concentration. Loop formation probability depends strongly on the distance between the binding sites and contrary to what is observed in cells, our data show that CTCF does not favor any particular binding site orientation *in vitro*. Endogenous CTCF from nuclear extracts also loop DNA specifically. Our results indicate that the preferred looping orientation observed *in vivo* likely results from additional regulatory looping factors.

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– 5. Protein-nucleic acid interactions –

P-104

Dynamic proofreading in bacterial DNA polymerase IIIH. L. Gahlon¹, G. Zhao³, R. Fernandez-Leiro³, M. H. Lamers³, D. Rueda^{1,2}¹Department of Medicine, Imperial College London, UK; ²London Institute of Medical Sciences, MRC, UK; ³Laboratory of Molecular Biology, MRC, UK

High fidelity DNA replication is important for maintaining genome stability. Errors during replication can establish mutations that can lead to diseases like cancer. DNA polymerases contain 3' to 5' proofreading activity that allows for removing misinserted nucleotides during DNA replication. During proofreading the primer strand migrates from the polymerase domain to the exonuclease domain where the error is removed. DNA polymerase III (DNA Pol III) is the replicative polymerase in *E. coli*. It can synthesise up to 1000 bp/s in its holoenzyme form. The goal of this work is to understand the dynamics of proofreading at single-molecule resolution. For this, we have developed a single-molecule FRET assay to monitor the active site switching dynamics during proofreading with several mismatches and an abasic site analog paired opposite A. Single-molecule FRET data will be presented that measure the binding kinetics for both wild type and a DNA Pol III mutant with altered proofreading activity. Transition density plots will be presented that demonstrate the conformational dynamics for DNA Pol III in the presence of varying DNA errors. Overall, our work is important as it provides insight into the mechanism that ensures high fidelity DNA synthesis.

P-106

Early stage of large ribosomal subunit assembly in E.coli: a single molecule studyL. Geffroy, T. Bizebard, U. Bockelmann
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In vitro reconstitution studies have shown that ribosome assembly is a highly cooperative process that starts with the binding of a few ribosomal (r-) proteins to rRNA, but how these early binders act is unknown. This work focuses on the initial phase of the assembly of the large subunit of the *E. coli* ribosome, which involves 23S rRNA, five r-proteins and assembly helper proteins.

We prepared a 79 nucleotide-long region of 23S rRNA encompassing the binding sites of the early binders L24 and L4 (proteins supplied by the group of Takuya Ueda, University of Tokyo). Force signals are measured in a dumbbell configuration using a dual-beam trap. We unwind the rRNA fragment without added protein, or in the presence of either L4 or L24 or both.

Without protein, the rRNA fragment unfolds by a sequence of steps that involve three intermediates. With L4 and/or L24, the same intermediates are observed but they unfold at higher force. For instance, the most probable unfolding force of the second intermediate is 10.7, 13.3, 14.9 and 16.4 pN, without protein or in the presence of L4, L24 or both, respectively. We localise the binding sites of the two r-proteins on rRNA within ± 5 nucleotides and observe pronounced cooperativity in their binding. We conclude that L4 and L24 act in concert to mechanically stabilise this region of 23S rRNA.

P-105

High precision single-molecule FRET reveals reversible transitions in nucleosomes on the microsecondA. Gansen¹, S. Felekyan², R. Kühnemuth², K. Lehmann¹, K. Tóth¹, C. A. M. Seidel², J. Langowski¹¹DKFZ, Div. Biophysics of Macromolecules, Heidelberg, Germany; ²Heinrich-Heine-Universität, Inst. für Physikalische Chemie, Düsseldorf, Germany

Nucleosomes play a dual role in compacting the genome and regulating access to specific DNA sequences. To unravel the underlying mechanism, we characterized structural and dynamic features of reconstituted mono-nucleosomes upon NaCl-induced destabilization, using multiparameter single-molecule FRET. Samples were labeled with donor/acceptor pairs on the DNA and on histone H2B. Species-selective fluorescence lifetime analysis, dynamic photon distribution analysis and fluorescence correlation spectroscopy allowed us to identify new intermediates during nucleosome opening, to describe their sub-millisecond transition kinetics and to develop a structural and kinetic model extending from microseconds to minutes. Opening proceeds through weakening of the interface between the H2A-H2B dimers and the (H3-H4)₂ tetramer on a 0.1 ms time scale, then by a slower two-step release of the dimers coupled to DNA unwrapping. Nucleosome opening and detachment of histone dimers proceed asymmetrically, determined by the DNA sequence. Our model describes the complete sequence of intermediates from intact nucleosome to free dimers/tetramers.

P-107

Acetylation and phosphorylation of TFAM have contrasting mechanisms for regulating TFAM-DNA interactM. Hashemi Shabestari¹, G. A. King¹, W. H. Roos^{2,1}, C. K. Suzuki³, G. J. L. Wuite¹¹Department of Physics, Vrije Universiteit Amsterdam, Netherlands; ²Zernike Institute, Rijksuniversiteit Groningen, Netherlands; ³Department of Biochemistry and Molecular Biology, New Jersey, USA

TFAM is a multifunctional protein that orchestrates mitochondrial DNA compaction, transcription and replication. While Post-translational modifications, such as TFAM phosphorylation and acetylation are thought to regulate these processes, the regulation mechanism is not understood. By single-molecule manipulation and fluorescence microscopy, we investigate the effect of TFAM phosphorylation and acetylation on DNA binding affinity. We determined the binding affinity of TFAM to DNA and the extent of TFAM induced DNA compaction. We demonstrate that phosphorylation and acetylation of TFAM do not alter its ability to compact DNA, but lower the binding affinity to DNA. Furthermore, we reveal an increase in the unbinding rate of TFAM from DNA upon phosphorylation. This indicates that the reduced binding affinity of TFAM to DNA when phosphorylated is partially due to the higher off-rate of phosphorylated TFAM. Conversely, the unbinding rate of TFAM from DNA remains unaffected by acetylation. We relate the lower binding affinity of acetylated TFAM to a decrease in the on-rate of the protein. These findings indicate that phosphorylation and acetylation can regulate TFAM function.

Posters

– 5. Protein-nucleic acid interactions –

P-108

TRBP and PACT pose stoichiometric questions for Dicer complex assemblyA. P. Heyam¹, M. P. Benoit², L. Imbert², A. Palencia³, J. Perard⁴, C. Ebel², D. Lagos^{1,5}, J. Boissbouvier², M. J. Plevin^{1,6}¹Dept. of Biology, University of York, UK; ²Institut de Biologie Structurale, Grenoble, France; ³European Molecular Biology Lab., Grenoble, France; ⁴Unit of Virus Host-Cell Interactions, Univ. Grenoble Alpes, France; ⁵Centre for Immunity & Infection, Hull York Medical School, Univ. of York, UK; ⁶York Structural Biology Lab., Univ. of York, UK

The RNase III enzyme Dicer catalyses a key step in microRNA (miRNAs) biogenesis. Dicer associates with two homologous double-stranded (ds) RNA-binding proteins, TRBP and PACT. Both proteins comprise three dsRNA-binding domains (dsRBDs) connected by flexible linkers. The two N-terminal domains are canonical dsRBDs, which bind dsRNA, while the C-terminal dsRBD is non-canonical and interacts with proteins, including Dicer.

Much remains unknown about the role of TRBP and PACT in the assembly and function of the Dicer complex. PACT and TRBP are reported to form homodimers as well as TRBP-PACT heterodimers, but their dimerisation properties are poorly understood and have yet to be rationalized in the context of Dicer complex stoichiometry and assembly.

We have investigated two aspects of TRBP and PACT stoichiometry. Using structural biology approaches, we have determined that the non-canonical dsRBD of TRBP and PACT homodimerises via an unusual but conserved asymmetric mechanism. We have also shown that two molecules of TRBP associate with miRNA precursors. In both cases, the formation of protein/dsRNA or homomeric protein/protein complexes occludes surfaces required for interactions with Dicer. Our results will be discussed in the context of Dicer processing of precursor miRNAs.

P-110

Sequence-based identification of protein-metal ions binding sitesX. Hu¹, D. Qiwen²¹Inner Mongolia University of Technology, China; ²East China Normal University, China

Metal ions play an important role in the biological life process. More than one-third of the protein structure contains metal ions. Therefore, it is particularly important to identify the metal ion-binding residues in the protein.

In this paper, the binding sites are predicted for seven metal ion (Cu²⁺, Fe²⁺, Fe³⁺, Ca²⁺, Mg²⁺, Mn²⁺ and Co²⁺) extracted from the BioliP database. The binding residues were identified by matrix scoring algorithm, the overall Matthews correlation coefficients (MCC) are 0.77, 0.72, 0.65, 0.40, 0.38, 0.53 and 0.60, respectively.

The performance was improved by using SVM algorithm inputted with the sequence information such as amino acid component, hydrophilicity and hydrophobicity, surface accessibility, and structure information such as secondary structure and solvent accessibility. The overall MCCs are 0.93, 0.83, 0.76, 0.50, 0.51, 0.66 and 0.66 respectively. We also used Random Forest (RF) algorithms with different features and data samples. Experimental results show that RF has good effect on the prediction of high-dimensional features of big samples where SVM has the advantage of predicting the optimization of characteristic parameters of small samples.

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P-109

Crystal structure of primosomal loader protein DnaB involved in DNA replication restart

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The Gram-positive bacterial DnaB primosomal protein plays a key role in DNA replication and restart as a loader protein for the recruitment of replisome cascade proteins. The DnaB protein is composed of three structural domains. However, structural evidence for how DnaB functions at the atomic level is lacking. Here, we report the crystal structure of DnaB, encompassing the N-terminal and middle domains (residues 1-300), from *Geobacillus stearothermophilus* (*GstDnaB*₁₋₃₀₀) at 2.9 Å resolution. Our structure shows that *GstDnaB*₁₋₃₀₀ forms a tetramer with two basket-like architectures which is consistent with solution studies using analytical ultracentrifugation. Furthermore, our results from both GST pull-down assays and analytical ultracentrifugation show that *GstDnaB*₁₋₃₀₀ is sufficient to form a complex with PriA, the primosomal re-initiation protein. Moreover, with the aid of small angle X-ray scattering (SAXS) experiments, we also determined the structural envelope of full-length DnaB (*GstDnaB*_{FL}) in solution. The SAXS studies show that *GstDnaB*_{FL} has an elongated conformation and the protruding density envelopes originating from *GstDnaB*₁₋₃₀₀ could completely accommodate the C-terminal domain (residues 301-461) of *GstDnaB*. Taken together, our findings provide insight into the functional role of DnaB primosomal protein in relation to DNA replication.

P-111

Remastering the specific DNA recognition by DNA primaseS. Ilic¹, A. Afek², D. Lukatsky¹, R. Gordan², B. Akabayov¹¹Ben-Gurion University of the Negev, Israel; ²Duke University, USA

The recognition sequence for DNA primase of bacteriophage T7 comprises a specific trimer sequence 5'-GTC-3', however, the principles that govern the probability to initiate RNA primer synthesis on genomic DNA are not known.

Using a combination of high-throughput protein-DNA binding microarray together with biochemical analysis, in a method we designated as High-throughput DNA Primase genomic profiling (HPgen) we identified DNA recognition features that contribute to primer synthesis by DNA primase but extend beyond the known trimer recognition site. We have designed microarrays with thousands of synthetic DNA sequences that cover wide-range of possibilities at the flanking sequences with respect to the included recognition site. The DNA sequences were incubated with primase under suitable conditions that allow strong binding to the DNA. The microarrays were scanned to comprehensively determine the binding profiling over a wide range of binding affinities, and these results were further examined using low throughput primer synthesis assay performed by T7 DNA primase. Using HPgen we show the significant influence of different flanking sequences on primer synthesis efficiency and DNA recognition of DNA primase. This method can be applied to other enzymes that recognize specific DNA sequences.

Posters

– 5. Protein-nucleic acid interactions –

P-112

***In vivo* compaction dynamics of bacterial DNA: A fingerprint of DNA/RNA demixing?**

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The volume occupied by unconstrained bacterial DNA in physiological saline solutions exceeds 1000 times the volume of prokaryotic cells. Still, the DNA is confined to a small region of the cell called the *nucleoid*. This is puzzling because bacterial DNA is not delimited by a membrane, in contrast with eukaryotic cells. There is still no general agreement on the mechanisms leading to the compaction of the DNA inside the nucleoid. However, advances in *in vivo* sub-wavelength resolution microscopy have recently allowed very detailed observations, which indicate that the size of the nucleoid varies greatly with several factors. I will argue that these observations provide converging indications in favor of a model that describes the cytosol as an aqueous electrolyte solution containing several macromolecular species, where demixing and segregative phase separation occur between DNA and RNA. I will also point out that crowding may play a crucial role by constraining macromolecules to feel electrostatic interactions in spite of the strong screening exerted by electrolyte species. This synergy ultimately favors stronger DNA/RNA demixing and nucleoid compaction.

References :

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P-113

DNA binding fluorescent proteins for visualizing large DNA molecules

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Fluorescent proteins that also bind DNA molecules are useful reagents for a broad range of biological applications because they can be optically localized and tracked within cells, or provide versatile labels for *in vitro* experiments. We report a novel design for a fluorescent, DNA-binding protein (FP-DBP) that completely ‘paints’ entire DNA molecules, whereby sequence-independent DNA binding is accomplished by linking a fluorescent protein to a variety of DNA binding peptides or proteins. Importantly, this ubiquitous binding motif enables fluorescent proteins to confluent stain DNA molecules and such binding is reversible. These proteins offer useful robust advantages for single DNA molecule studies: lack of fluorophore mediated photocleavage and staining that does not perturb polymer contour lengths. Accordingly, we demonstrate confluent staining of naked DNA molecules presented within microfluidic devices, or localized within live bacterial cells.

P-114

Iterative homology checking and non-uniform stepping during RecA-mediated strand exchange

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Recombinase-mediated homologous recombination (HR) in which strands are exchanged between two similar or identical DNA molecules is essential for maintaining genome fidelity and generating genetic diversity. By using single-molecule approaches to catch transient intermediates in RecA-mediated HR with different degrees of homology, we show that (i) the expansion proceeds with step sizes of multiples of 3 bp, (ii) the step sizes follow wide distributions that are similar to that of initial alignment lengths, and (iii) each distribution can be divided into a short-scale and a long-scale part irrespective of the degree of homology. Our results suggest an iterative mechanism of strand exchange in which ssDNA-RecA filament interrogates double-stranded DNA using a short tract (6–15 bp) for quick checking and a long tract (>18 bp) for stringent sequence comparison.

P-115

Crystal structures of cyanine fluorophores stacked onto the end of double-stranded RNA

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University of Dundee, UK

Fluorescence resonance energy transfer (FRET) is an important source to determine the approach between two molecules within several nanometers. Cy3 and Cy5 are one of the most commonly used donor-acceptor pairs of fluorophores in the single-molecule studies. The accurate distance between the Cy3 and Cy5 requires the understanding of the position and orientation of the fluorophores. In our previous study, we have determined the structures of Cy3 and Cy5 attached to the 5'-terminus of double-helical DNA respectively by NMR. Here, We developed a co-crystallization system using RNA bounded T. Thermophilus L5 protein to study the structure of Cy3 and Cy5 attached to the 5'-terminus of RNA helix. The result shows Cy3 and Cy5 could also stack onto the end of the RNA duplex but distinct from the stacking onto the DNA. The stability of the stacking is also sequential and environmental selected.

Posters

– 5. Protein-nucleic acid interactions –

P-116

The structural and catalytic study of the novel twister ribozyme and TS ribozyme

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Ribozymes are RNA molecules that act as chemical catalysts. Nucleolytic ribozymes are a class of self-cleavage ribozymes with the mechanism of nucleotidyl transfer. They might reflect the most fundamental chemical reaction during the start of life, and are also good candidates for therapeutic use. Recently, four new members of nucleolytic ribozymes were discovered. Here we reported the novel structures of the two new members (twister ribozyme and TS ribozyme) and the mechanism of their actions at atomic level. The twister ribozyme RNA adopts a previously uncharacterized compact fold based on a double-pseudoknot structure, with the active site at its centre. The strongly conserved G33 acts as general base in the general acid-base-catalysed cleavage reaction. The adenine immediately 3' to the scissile phosphate acts as a general acid. Very unusually, the N3 of A1 is the proton donor to the oxyanion leaving group. Besides, our high-resolution structure of TS ribozyme indicates its connectivity of the helices is strikingly similar to a double-pseudoknot arrangement as twister, yet topologically not equivalent. A catalytic metal ion plays an important role in TS, which is absent in twister. Our structural and mechanical study of these ribozymes reveals new insights into RNA catalysts and indicates the promising therapeutic application of these RNA.

P-118

Condensation of DNA mediated by the amyloidogenic C-terminal domain of HfqA. Malabirade¹, K. Jiang², A. Diaz-Mendoza³, J.-F. Berret³, V. Arluison^{1,3}, J. R. C. van der Maarel²

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Hfq is a bacterial protein that is involved in several aspects of nucleic acids metabolism. It has been described as one of the nucleoid associated proteins shaping the bacterial chromosome, although it is perhaps better known to influence translation and turnover of cellular RNAs.

Here, we explore the role of *Escherichia coli* Hfq's C-terminal domain in the compaction of double stranded DNA. Various experimental methodologies, including fluorescence microscopy imaging of single DNA molecules confined inside nanofluidic channels, AFM, ITC, and electrophoretic mobility assays have been used to follow the assembly of the C-terminal and N-terminal regions of Hfq on DNA.

Results highlight the role of Hfq's C-terminal arms in DNA binding, change in mechanical properties of the double helix and compaction of DNA into a condensed form. The propensity for bridging and compaction of DNA by the C-terminal domain is presumably related to aggregation of bound peptide as in amyloid fibril formation. The formation of this functional amyloid may have implications for protein binding related gene regulation.

P-117

Nanotensioners for FRET assay of helicases with one-nucleotide resolution

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Single-molecule FRET is widely used to study helicases by detecting distance changes between a fluorescent donor and an acceptor anchored to overhangs of a forked DNA duplex. However, it has lacked single-base pair (1-bp) resolution required for revealing stepping dynamics in unwinding because FRET signals are usually blurred by thermal fluctuations of the soft overhangs. We designed nanotensioners in which a short DNA is bent to exert a force on the overhangs, just as in optical/magnetic tweezers. The strategy improved the resolution to 0.5 bp, high enough to uncover the differences in DNA unwinding by yeast Pif1 and *E. coli* RecQ whose molecular mechanisms cannot be differentiated by currently practiced methods. We found that Pif1 follows a 1-bp-stepping mechanism, while RecQ breaks 1 bp at a time but sequesters the nascent nucleotides and releases them randomly. A general model with three kinetic parameters, namely, a base-pair breaking rate, a 3'-tail releasing rate and a 5'-tail releasing rate, can phenomenologically describe the different mechanisms.

P-119

Partial intercalation kinks the DNA: Mechanism of protein-DNA intercalation

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In this talk, we will discuss the origin of kink formation in DNA observed for some transcription factor (TF) protein-DNA complexes. We investigate the molecular origin of the DNA kinks using small molecule intercalation pathway, crystallographic analysis, and free energy calculations involving four different transcription factor (TF) proteins and their complexes with DNA. We find that although protein binding may bend the DNA, it alone is not sufficient to kink it. We will show that partial, not complete, intercalation is required to form kink at a particular place in DNA. We find that while amino acid alone can induce the desired kink through partial intercalation, protein provides thermodynamic stabilization of the kinked state in TF-DNA complexes.

Continuing this, we investigated the complete mechanism of intercalation of some of these transcription factor proteins into DNA to understand how the kinking and bending of the DNA are interrelated along the path of the intercalation process with the broader goal to understand the role of DNA bends and kinks in protein-DNA interactions.

Posters

– 5. Protein-nucleic acid interactions –

P-120

Structural heterogeneity of the attC integron recombination site promotes strand selectivityA. Mukhortava¹, M. Poegel^{1,4}, M. S. Grieb¹, A. Nivina^{2,3}, C. Loot^{2,3}, D. Mazel^{2,3}, M. Schlierf¹¹B CUBE – Center for Molecular Bioengineering, TU Dresden, Germany; ²Institut Pasteur, Bacterial Genome Plasticity Unit, France; ³CNRS UMR3525, France; ⁴Max-Planck-Institut für Biochemie, Germany

Integrans are the major genetic tools for the acquisition of multiple antibiotic resistances by bacteria. Resistance genes are embedded in cassettes consisting of promoterless genes flanked by two recombination sites called *attC*. The tyrosine recombinase IntI recognizes a single-stranded *attC* site folded in a hairpin and recombines it with the integron platform, ensuring the expression of the encoded cassette. Interestingly, IntI shows a remarkable strand selectivity for the bottom strand *attC* hairpin (*attC_{bs}*). However, little is known on how IntI distinguishes the *attC_{bs}* from the top strand (*attC_{ts}*), which have complementary sequences and thus are expected to be structurally very similar.

Here, we used single-molecule optical tweezers to study the molecular structure of two complementary *attC_{bs}* and *attC_{ts}* hairpins. Both hairpins unfolded at surprisingly low forces (5 pN) via intermediate states. The force-induced unfolding revealed the existence of two structurally distinct, but energetically similar conformations for both hairpins. The predominant conformation of *attC_{bs}* exposed the IntI binding site, while the predominant conformation of *attC_{ts}* buried it. Mutational studies revealed that this conformational heterogeneity is governed by unpaired features of the hairpins.

P-122

A fluorescence polarization based assay for cap-binding proteinsA. Nowicka^{1,2}, R. Kasprzyk³, D. Kubacka¹, M. Warmiński¹, K. Mokrzejewska¹, J. Kowalska¹, J. Jemielity²¹Faculty of Physics, University of Warsaw, Warsaw, Poland; ²Centre of New Technologies, University of Warsaw, Warsaw, Poland; ³College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland

Cap-binding proteins play a key role in mRNA metabolism by interacting with the cap structure (m⁷GpppN_n) present at the 5' mRNA end or its hydrolysis products. The cap-binding proteins are specific regulators of mRNA metabolism and their activity is under tight control in the cells. Fluorescently labeled cap analogs are attractive probes to study activity of cap-binding proteins and consequently processes of gene expression.

Here we present, the set of fluorescently labeled cap analogs which were characterized as probes for fluorescence polarization method. Synthesized probes have attached carboxyfluorescein label to different position of cap analog: ribose moiety, phosphate group or nucleobase. Selected probes were employed for competition studies with translation factor 4E and DcpS enzyme. Both proteins are therapeutic targets: eIF4E has been found in many types of tumor cells where it induces selective increase in the translation of mRNAs and DcpS inhibition alters expression of SMN protein reducing symptoms of spinal muscular atrophy. Obtained EC₅₀ parameters for selected non-fluorescent ligands are in good agreement with literature data for both proteins.

P-121

Force induced off-target binding of CRISPR/Cas9 with single molecule resolutionM. D. Newton¹, R. Driessen², B. J. Taylor³, E. Cuomo³, D. Rueda¹¹Department of Medicine, Section of Virology and MRC London Institute of Medical Sciences Imperial College London, UK; ²Lumicks, De Boelelaan, 1085 Amsterdam, Netherlands; ³AstraZeneca, R&D Discovery Sciences, Cambridge, UK

CRISPR/Cas9 has become a widely used tool for sequence specific genome editing. However, current algorithms fail to accurately predict some of the off targets observed *in vivo*, highlighting a gap in our understanding of the mechanism by which CRISPR/Cas9 locates on/off targets. To address this, we have developed a single-molecule assay combining optical tweezers with fluorescent detection to monitor binding of labelled crRNA-SpCas9 complexes to stretched lambda DNA. At low forces, the complex binds tightly and specifically on a single target site, and cleaves the DNA in the presence of Mg²⁺. Stretching the DNA to higher forces reveals numerous off targets binding events, with dwell times ranging from a seconds to minutes depending location and force. Interestingly, the complexes repeatedly bind the same off-target sites, but these sites could not be explained by current prediction algorithms. Using eGFP labelled hRPA (a ssDNA binding protein), we show that the observed off-target binding is not due to the presence of DNA nicks, large DNA bubbles or ssDNA, suggesting that off-target binding requires both DNA strands. We anticipate that these observations will help establish new rules for off-target site prediction, which could aid future Cas9 experimental design.

P-123

Order and disorder prediction in N-terminal domain of the DNA partitioning protein IncC using NMRM. F. Rehman¹, M. Jeeves², A. Gautam¹, S. White¹, C. Thomas¹, D. Scott³, E. Hyde¹¹School of Biosciences, Univ. of Birmingham, Edgbaston, Birmingham, UK; ²Inst. of Cancer and Genomic Sciences, Univ. of Birmingham, UK; ³School of Biosciences, Univ. of Nottingham, Sutton Bonington Campus, Leicestershire, UK

Plasmid DNA partitioning is a crucial process for the transfer of at least a single copy of plasmid to the daughter cells during cell division in bacteria. With low-copy number plasmids the partitioning process involves; a DNA-binding protein, a centromere-like DNA site, “ParS” and a ParA family protein ATPase. In low copy number, IncP1 plasmid, RK2, IncC protein from the is a Par A protein and, unusually express in two different lengths. The longer form is named IncC1 and the shorter one, in which 105 amino acids at the N-terminal are missing, is IncC2. IncC2 is similar in structure to ParA proteins involved in chromosomal DNA partitioning. We are currently studying the N-terminal region of IncC1 to allow us to differentiate the functions of two different forms of IncC to elucidate the possible roles of this N-terminal Domain. Using Circular Dichroism, Small Angle X-ray Scattering and ¹H¹⁵N¹³C NMR, this domain has been found to be intrinsically disordered. We have used several techniques including carbon detected NMR, EMSA, micro-scale thermophoresis (MST), fluorescence anisotropy and circular dichroism to get the secondary structure information for IncCNTD. It appears to bind DNA weakly, possibly non-specifically and is intrinsically disordered.

Posters

– 5. Protein-nucleic acid interactions –

P-124

NMR and biophysical studies of G-quadruplex DNA within the KRAS proto-oncogene promoter regionG. F. Salgado¹, J. Marquevielle¹, P. Notelet¹, L. A. Yatsunyk², A. Guedin¹, A. Kerkour¹, J.-L. Mergny¹¹Université de Bordeaux, Inserm, CNRS, European Institute of Chemistry and Biology., France; ²Swarthmore College, 500 College Ave, Swarthmore 19081, PA, USA, USA

The role of G-quadruplexes (G4) which can coexist with canonical duplex DNA in the human genome is still largely unknown. Although G4s are found throughout the entire genome, much of the attention have been invested in promotor regions of disease-related genes. The human KRAS proto-oncogene contains a nuclease hypersensitive element (4) located upstream of the major transcription start site. This region regulates transcription of KRAS and it was proposed as a new target for drug development. The knowledge of the detailed structure of this target is crucial for the development of new effective drugs. In this study, we report a high-resolution NMR structure of the G-rich element within the KRAS NHE, and their interaction with small ligands. The G-rich elements forms a parallel structure with three G-quartets connected by a four-nucleotide loop, two one-nucleotide double-chain-reversal loops and a thymine bulge. The loops of different lengths and the presence of a bulge between G-quartets are structural elements that can potentially be targeted by small ligands that would further stabilize the structure. In addition, we explore the polymorphism of G-quadruplexes structures within the promotor region. Consequently, our work suggests an alternative route for the development of anticancer agents that could regulate KRAS expression.

P-126

The forces stabilising the DNA duplexP. Vaitiekunas¹, P. L. Privalov¹, C. Crane-Robinson²¹Department of Biology, Johns Hopkins University, Baltimore, USA; ²School of Biology, University of Portsmouth, Portsmouth, UK

Understanding what keeps the strands of B-form DNA together needs accurate values for the enthalpy and entropy of association/dissociation and for the accompanying heat capacity change that defines their temperature dependence. The relative success of T_m prediction protocols suggests our knowledge is adequate but the ΔH and ΔS values in the literature are actually wide of the mark: their predictive capability is due to compensating errors. To clear up the mess we combined DSC & ITC of short (9–15bp) duplexes of variable sequence. The results of strand association/dissociation experiments are as follows. There is an identical heat capacity increase of +0.13 kJ/K.mol-bp for dissociating both AT and CG pairs, the result of hydrating the hydrophobic bases. The heat of dissociating a CG pair is *less* than for an AT pair, despite its extra H-bond. This means that an AT-rich duplex melts at lower temperatures because the entropy of dissociating an AT pair is much greater than that of a CG pair. This increased entropy comes from loss of highly ordered water bound to AT pairs in the minor groove. Comparison of CG-rich and AT-rich duplexes shows that the sequence-dependence of T_m values—the ‘nearest-neighbour’ effect—is an AT property and a consequence of their specific hydration.

P-125

Characterization of a second Single Stranded DNA Binding protein from *Mycobacterium smegmatis*A. Singh¹, U. Varshney², M. Vijayan¹¹Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India; ²Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India

In addition to the canonical Single Stranded DNA Binding (SSBa) protein, many bacterial species, including mycobacteria, have a paralogous SSBb. The biological function of SSBb is still ambiguous. We have structurally and biochemically characterized SSBb from *M. smegmatis* (*MsSSBb*). Unlike other organisms where SSBbs are shorter than SSBa, *MsSSBb* is of similar length as *MsSSBa*. The protein crystallizes in hexagonal space group $P6_522$ ($a = b = 73.61$ Å, $c = 216.21$ Å), with half a tetrameric molecule in the asymmetric unit of a cell. The tertiary and quaternary structure of the DNA binding domain of *MsSSBb* is similar to that observed in *MsSSBa*, retaining the swapped β -strand ‘hook’. Solution studies indicate that *MsSSBa* is more stable towards thermal and chemical denaturation than *MsSSBb*. *MsSSBa* has a two-fold higher DNA binding affinity than *MsSSBb*. However, in other organisms, SSBb’s usually have 5–20 fold higher affinity than their respective SSBa’s. The expression levels of *ssbB* gene increased by approximately 2 and 7 fold in UV and hypoxic stress, while simultaneously the levels of *ssbA* expression declined. A direct physical interaction of *MsSSBb* and RecA was established, indicating a probable role of *MsSSBb* in recombination repair during stress.

P-127

Target discrimination and dynamic sequence search by the telomeric protein TRF1

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Target search as performed by DNA-binding proteins is a complex process, in which multiple factors contribute to both thermodynamical discrimination of the target sequence from overwhelmingly abundant off-target sites and kinetic acceleration of dynamic sequence interrogation. TRF1, the protein that binds to telomeric tandem repeats, faces an intriguing variant of the search problem where target sites are clustered within short fragments of chromosomal DNA. In this study, we use extensive (>0.5 ms in total) MD simulations to study the dynamical aspects of sequence-specific binding of TRF1 at both telomeric and non-cognate DNA. For the first time, we describe the spontaneous formation of a sequence-specific native protein-DNA complex in atomistic detail, and study the mechanism by which proteins avoid off-target binding while retaining high affinity for target sites. Our calculated free energy landscapes reproduce the thermodynamics of sequence-specific binding, while statistical approaches allow for a comprehensive description of intermediate stages of complex formation.

Posters**– 5. Protein-nucleic acid interactions –****P-128****Fluorescence & light scattering approaches to study mechanisms of translation initiation on LL mRNAs**

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Translation initiation is the first step of protein synthesis, whereby the mRNA start codon is encountered by the ribosome with the assistance of initiation factors (IFs). The canonical translation initiation in bacteria (30S-initiation pathway) involves recognition by the 30S ribosomal subunit of the Shine-Dalgarno sequence from the mRNA 5' leader. Together with the 30S subunit, initiator fMet-tRNA^{fMet}, initiation factors IF1-IF3 and GTP, a leader-containing (LC) mRNA forms the 30S initiation complex (30S IC), which is transformed to the 70S IC complex after 50S subunit joining, GTP hydrolysis and release of IFs. The latter complex binds ternary complex (TC) and forms peptide bonds.

Here we demonstrate mechanisms of translation initiation on leaderless (LL) mRNAs studied by fluorescence and light scattering. We have found that 30S subunits can initiate LL mRNA translation if IF3 is missing. In contrast, LL mRNAs can be initiated by 70S ribosomes provided that IF3 is present. Remarkably, initiation on LL mRNAs via the non-canonical 70S-initiation pathway is more efficient and stimulated by IF1, although IF1 is not required. Because LL mRNAs can be formed by the stress-induced toxin-antitoxin bacterial systems, our research provides insights into stress adaptation in bacteria.

Posters

– 6. Protein structure to function –

P-129 (O-40)

Bacterial surface-layer-protein assemblies at atomic scale

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S-layers are (glyco)protein coats displayed on the external surface of many bacterial and archaeal species. As the outermost layer, the S-layer is in direct contact with the environment and thus involved in e.g. adhesion to epithelial cells and intestinal components. S-layers however remain poorly understood, primarily due to lack of structural knowledge.

We characterized the bacterial surface layer proteins SbsC from *Geobacillus stearothermophilus* and SlpA of *Lactobacillus acidophilus* and *L. amylovorus*. Several soluble fragments as well as full-length proteins assembling to 2D-crystals were produced. To elucidate the structure of the complete S-layers, an integrative structural biology approach combining X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR), mass spectrometry and electron microscopy has been applied. To additionally characterize the binding of S-layer proteins to the bacterial cell wall we performed isothermal titration calorimetry (ITC) experiments. The obtained results allow us to learn more about the cell wall attachment and self-assembly formation on atomic scale.

P-131 (O-42)

A tri-ubiquitin bridges two ABIN2 dimers to form a higher-order signaling complex

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Poly-ubiquitin chains are important signaling molecules that were assembled by the ubiquitination process in order to drive different linkage-specific signaling pathways through subsequent protein-protein interaction with downstream signaling molecules. For example, K63 and linear poly-ubiquitin chains play an important role in NF- κ B activation pathway. A20 could down regulate the NF- κ B activation pathway by interacting and degrading the poly-ubiquitin chains. The interaction is mediated by ABIN2, which has both A20- and linear poly-ubiquitin interacting domains. How a poly-ubiquitin chain interacts with signaling molecules remains largely unknown. Here we report the crystal structure of an ABIN2:tri-Ub complex. ABIN2 has a primary and a secondary linear-Ub binding site. Surprisingly, a tri-Ub molecule could simultaneously interact with two ABIN2 dimers, in which the ubiquitins form a helical trimer when bridging two hABIN2 dimers. Our studies suggest the formation of a higher-order complex between linear poly-polyubiquitin chain, ABIN2, and A20.

P-130 (O-41)

Observation of water-channel opening of cytochrome c oxidase by time-resolved XFEL crystallography

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Bovine heart cytochrome c oxidase (CcO) is a large membrane enzyme (210 kDa) that catalyzes O₂ reduction to water, coupled with proton pump across the mitochondrial inner membrane. The enzyme includes a water channel and a hydrogen-bond network in tandem as a proton-pump pathway. After the O₂ reduction at the active center consisting of Fe_{a3} and Cu_B, CcO receives four electron equivalents from cytochrome c. Each of the electron transfer processes is coupled with pumping of one proton equivalent, during which the water channel is closed to prevent proton back leak. Recently, it was revealed that binding of CO (O₂ analogue) to Fe_{a3} induces a bulge (unpaired backbone C=O) formation at Ser382 in Helix-X, which closes the water channel. To elucidate the water-channel gating mechanism, here, we developed an instrument for time-resolved X-ray crystallography using an X-ray free electron laser at SACLA, and investigated the structural dynamics upon CO photo-dissociation from Fe_{a3}. As a result, we successfully observed the water channel opening processes, accompanied with transient CO binding to and dissociation from Cu_B. The water-channel gating mechanism based on a dynamic interaction between the active center and Helix-X will be discussed.

P-132

Molecular insights for resistance conferring mutations in competitive inhibition

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While antibiotic resistance is an organism scale phenomenon requiring coordinated arrangements on a series of time and length scales, that acquiring point mutations on enzymes leads to resistant strains implies significant changes at the atomistic scale. We report the mechanisms by which the enzyme dihydrofolate reductase fights back the increased amounts of trimethoprim introduced in the environment. The accumulated resistance conferring mutations are determined via the experimental “morbidostat” setup. The changes in the enzyme structure and dynamics are monitored by extensive molecular dynamics simulations and free energy difference calculations on 48 variants. The emergence of antibiotic resistance is shown to occur in an order such that the first mutants stabilize substrate binding by establishing additional favorable contacts, followed by mutants that further and synergistically act on the same site. Additional mutants are effective on loosening inhibitor binding, eventually leading to a “dead” enzyme. Enthalpy-entropy compensation emerges as a frequently acquired strategy by the mutating protein. The results are corroborated by thermodynamic and kinetic measurements. The findings will guide future work in developing new and potent inhibitors from existing drug derivatives.

Posters

– 6. Protein structure to function –

P-133

Biophysical characterization of hypochlorous acid modified human antithrombin

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Activated phagocytes initiate the production of hypochlorous acid (HOCl) which reacts with a wide variety of biomolecules including proteins. Antithrombin (AT) is involved in many critical bio-processes like blood coagulation and inflammation. Any alteration in its structure and function may lead to various diseases.

In this study, different biophysical techniques were used to check the effects of HOCl on conformation of AT. Incubation of AT with various concentrations of HOCl showed polymerization followed by fragmentation in polyacrylamide gels. Furthermore, TEM confirmed polymerization. Compared to the unmodified AT, a gradual increase in hyperchromicities in absorbance spectra and a significant gradual decrease in fluorescence intensities in emission spectra of modified AT was observed with increasing concentrations of HOCl. Alteration in secondary structure was monitored by Far-UV CD spectroscopy. Compared to the unmodified AT, HOCl-modified counterparts showed gain in fluorescence intensities in ANS binding experiment indicating more and more exposure of hydrophobic patches on the surface of AT upon HOCl treatment.

The results suggest profound effects of HOCl on conformation of AT that may have implications in various diseases.

P-135

Iron core structure in ferritin and its pharmaceutical analogues studied by Mössbauer spectroscopy

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Iron storage protein ferritin and its pharmaceutical analogues (iron-polysaccharide complexes) contain iron in the form of nanosized ferric hydroxide cores (ferrihydrite and akaganéite, respectively). The structure of these iron cores is not clear yet in spite of numerous studies. We consider the results of the study of human liver ferritin and its iron-polymaltose analogues Ferrum Lek, Maltofer® and Ferrifol® using Mössbauer spectroscopy with a high velocity resolution in the temperature range 295–90 K. Application of a consistent heterogeneous iron core model to fit the Mössbauer spectra of ferritin and its analogues demonstrated the possibility to consider at least five iron core regions/layers with small variations of hyperfine parameters which can be related to more or less close packing in different regions/layers. Some observed features of Mössbauer parameters temperature dependences can be related to low-temperature structural rearrangement in the iron core with the change of the relative parts of more or less close packed iron core regions/layers. This work was supported by the Ministry of Education and Science of the Russian Federation (the Project # 1959).

P-134

Homology modelling of the human D2 dopamine receptor protein

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D2 dopamine receptors are mainly found in the central nervous system (CNS). D2 receptors couple to one of the G protein family; Gi/o. The function of Go protein is not known so clearly. Therefore, the details of the probable interaction between the D2 receptor and Go protein would be crucial for revealing the role of the Go protein in the CNS. But, a complete and high resolution structure of the D2 protein receptor is not available yet.

D2 receptor is a membrane protein and it could be very difficult to obtain tertiary structure of such proteins. In case of D2 receptor, third intracellular loop (ICL3) is another drawback. ICL3 is a very long loop and there are no homologous tertiary structure available for it.

In this study, a complete tertiary structure of the D2 receptor protein was modelled using homology modelling and molecular dynamics (MD) simulation in two steps. At first step, only tertiary structure of ICL3 was modelled. At second step, the complete structure of the D2 receptor protein was obtained by homology modelling using predicted ICL3 structure as one of the template structure. At the end of the simulations, overall GPCR topology was obtained and also the characteristic structural properties of the D2 receptor were observed in the predicted structure.

P-136

Biophysical study of a target bacterial protein using DLS, CD and fluorescence spectroscopy

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In this work we present the biophysical studies of the leucine aminopeptidase (LAP), a protein present in *Leptospira interrogans*. This protein is related to pathogenicity of the bacteria in animals and humans. The tests were performed using dynamic light scattering, circular dichroism and static fluorescence. LAPr-Li is self assembled as hexamer and its secondary structure consists of 44.7% α -helix and 11.6% for β -sheets. The hexamer with diameter of 15.2 nm and molecular weight 320 kDa. Fluorescence decay studies using charged and neutral quenchers and Stern-Volmer fitting revealed two different populations of tryptophan in negatively charged microenvironment. LAP present structural stability at 50°C in pH 8.5. It promotes flexibility and to be essential for enzymatic activity. The thermal denaturation curves (25–95°C) indicated structural stability at pH 3.0 and 5.0. At pH 8.0, 8.5 and 9.0 LAPr-Li was partially denatured resulting in lower values of ΔG^{25} . This study is fundamental to establish conditions of technological use and to use the enzyme as a target of drugs.

Posters

– 6. Protein structure to function –

P-137

Time-Resolved Crystallography from femtosecond laser driven X-ray plasma source in ELI beamlines

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The laser driven X-ray plasma source that is currently in a development stage at ELI-beamlines in Dolny Brezany near Prague (CZ), will deliver femtosecond X-ray pulses suitable for time resolved crystallography. The potential samples will be crystals from proteins possessing fast photo-cycles on sub-pico to nano and microseconds time scale. The generated X-ray pulses will have a repetition rate of 1 kHz, and intensity on the sample $\sim 10^6$ ph/pulse at 10.83 KeV. The scattered and diffracted by the crystal X-rays will be counted using a DECTRIS Eiger 1M area detector which operates at the same frame rate as the source, i.e. 1 kHz. Such setup can be combined with several pump probe lasers to study fast kinetic processes. Due to the interdisciplinary nature of the fields and of the ELI beamlines facility regular discussions between experts in high power laser-matter interaction and potential users, as well as young scientists, are organized.

B. Rus ; F. Batysta ; J. Čáp ; M. Divoký ; M. Fibrich, et al. "Outline of the ELI-Beamlines facility", Proc. SPIE 8080, 808010, 2011; doi:10.1117/12.890392

P-139

Structural characterisation of bacterial AdhE, a potential novel anti-virulence target

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The spiroosome-forming bifunctional alcohol-aldehyde dehydrogenase (AdhE) protein plays a role in the regulation of enterohaemorrhagic *Escherichia coli* virulence. Deletion of *adhE* results in a pleiotropic phenotype characterised by the suppression of the type three secretion system and the over-expression of non-functional flagella. Therefore AdhE is an attractive anti-virulence drug target and one of our goals is to determine its high-resolution structure of AdhE as a basis for structure-based drug design. Analytical ultracentrifugation shows that AdhE is polydisperse and self-associates into spiroosomes with an *s* ranging from 35 – 40 S. Two-dimensional class averages of electron micrographs of the protein reveal repeats within the spiroosome to be ≈ 75 Å apart in agreement with a Bragg peak at 78.5 Å observed by small-angle X-ray scattering. Due to its self-assembling behaviour we have focused on obtaining the high and low-resolution structure for the two domains separately. X-ray crystallography reveals that the high-resolution structure of alcohol dehydrogenase of AdhE agrees well with the low-resolution structure of the protein in solution. Current work is focused on the optimisation of crystal growth for the aldehyde dehydrogenase portion of AdhE.

P-138

Noncovalent PEGylation using lectin-glycopolymers interactionsP. Antonik¹, A. Eissa², A. Round³, N. Cameron², P. Crowley¹

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PEGylation, the covalent attachment of polyethylene glycol (PEG) to proteins, is a widely used technique to improve pharmacokinetics.^{1,2} The weakness of this method is that the polymer chains can diminish biological activity of the protein.^{1,2} Reversible, noncovalent PEGylation can potentially overcome this limitation. RSL (~ 29 kDa trimer) is a hexavalent fucose-binding lectin from the bacterium *Ralstonia solanacearum*³ that we characterized recently by NMR spectroscopy.⁴ Here, we present the interactions of RSL with a fucose-capped polyethylene glycol (Fuc-PEG). Using a combination of NMR spectroscopy, small angle X-ray scattering, size exclusion chromatography and native gel electrophoresis we demonstrate that RSL and Fuc-PEG form a high molecular weight protein-polymer conjugate. Moreover, we show that the assembly is reversible and the affinity for Fuc-PEG binding was estimated in the μ M range by isothermal titration calorimetry.

1. P. Caliceti *et al*, *Adv. Drug Deliv. Rev.*, **2003**, *55*, 1261

2. P. Kontos *et al*, *Chem. Soc. Rev.*, **2012**, *41*, 2686

3. N. Kostlanova *et al*, *J. Biol. Chem.*, **2005**, *280*, 27839

4. P. Antonik *et al*, *Biochemistry*, **2016**, *55*, 1195

P-140

Membrane activation of a bacterial GEF investigated by Molecular Dynamics with Excited Normal ModesE. Balog¹, B. Dudás², F. Peurois³, D. Perahia³, J. Cherfils³

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Arf GTPases are major regulators of most aspects of lipid and membrane traffic. They cycle between an inactive, cytosolic state bound to GDP, and an active, membrane-bound state bound to GTP. Guanine nucleotide exchange factors (GEFs) promote this intrinsically slow GDP to GTP exchange, resulting in an active membrane-bound conformation that interacts specifically with the downstream effectors. *Legionella pneumophila*, a bacterium that causes severe pneumonia (Legionnaire's disease), injects a large number of effectors that divert membrane traffic to establish a vacuole where it hides and replicates. One of these effector proteins is RalF, which diverts the host traffic by functioning as an illicit GEF to activate host Arf1 on the Legionella-containing vacuole. RalF is comprised of a Sec7 domain, which carries the GEF domain, and an autoinhibitory domain which is also responsible for the recruitment of RalF to the vacuole membrane. The structure of autoinhibited RalF has been determined, but the major conformational change needed to reach its active membrane-bound conformation has remained unknown. Using the method of Molecular Dynamics with Excited Normal Modes (MNeNM) here we present the mechanism of autoinhibition release and the membrane-bound open conformation of RalF.

Posters

– 6. Protein structure to function –

P-141

pH induced structural switch of CueR metal-loreulatory protein

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CueR regulates the intracellular level of Cu^I in bacteria by providing a sensitive and selective response to Cu^I (and Ag^I or Au^I), but not to double-charged transition metal ions (Zn^{II}, Hg^{II}). Understanding the details of the selective regulation may forward the design of molecules for selective metal ion binding or detection.

The function of CueR is based on the conformational change of the protein upon metal ion coordination, influencing the structure of the protein-bound DNA. Metal ion coordination occurs in a metal binding loop of the protein where two cysteine residues provide linear geometry around the metal ion. To obtain a deeper insight into the selective metal ion binding and regulatory function of the CueR protein, we investigated the effect of pH, the presence of mono and divalent metal ions, the promoter DNA sequence and combination of these on the relative proportion of secondary structure elements. The CD spectrum of the protein in the presence of Ag^I ions was different from the spectra recorded with Zn^{II}, Hg^{II} and Cd^{II} ions. Furthermore, decreasing the pH to 6.0 induced the transformation of the helix-rich structure into a β -sheet rich form. In order to reveal the effect of pH on the structure of CueR, we performed Molecular Dynamic simulations.

P-143

Intermolecular interactions of serum albumins in presence of metal ions

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Serum albumins (SA) are some of the most abundant proteins in the blood of mammals. The main function of SA is to transport different ligands and metal ions in particular. Such interactions might result in changes of protein structure and stability and can affect their aggregative ability. Our present work is devoted to studying interactions of Ca²⁺, Mg²⁺, Mn²⁺ and Cu²⁺ ions with human and bovine SA.

We have analyzed secondary structure of the proteins, size and the amount of dimers in solution, using a combination of FTIR spectroscopy in H₂O/D₂O solutions, dynamic light scattering, analytical ultracentrifugation. We have found that the presence of the metal ions stimulates intermolecular interaction in the protein solution. We have observed increased amount of the intermolecular β -structures in the SA/Me²⁺ complexes, compared to the pure proteins, which was attributed to the formation of the protein dimers.

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P-142

An investigation of effect of glycation on collagen fibrillar structure

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Glycation is a non-enzymatic reaction between sugars and proteins and is implicated in the pathogenesis of various diseases, including diabetes, neurodegeneration, cancer, and ageing. Long-lived proteins such as collagen are the most likely candidates to be affected by glycation reactions. Collagen is the most abundant and important extracellular matrix protein in the body. Collagen glycation has the effect of tissue stiffening. This work focuses on the effect of glycation on collagen fibrillar structure and the underlying mechanism of fibril stiffening. We have found that glycation causes disruption to the collagen banding and sub-banding by Transmission Electron Microscopy. Atomic force microscopy shows complete loss of the gap zone in glycated collagen. Kelvin force microscopy demonstrated that glycation causes change in the surface potential of collagen. Altogether, these different biophysical methods indicate that glycation causes change in molecular ordering in collagen fibrils. Furthermore, we examined the effect of collagen glycation on amyloid β aggregation, an extracellular deposit in Alzheimer's disease. We observed that the glycated collagen exacerbates the aggregation of amyloid β . This study gives insight into the link between ageing and Alzheimer's disease.

P-144

Allosteric communication in the heterodimeric ABC exporter TmrAB observed with PELDOR

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ATP-binding cassette (ABC) transport complexes use ATP binding and subsequent hydrolysis to translocate substrates across membranes. Here we investigated the heterodimeric ABC exporter TmrAB^[1] from *Thermus thermophilus* by electron paramagnetic resonance (EPR) spectroscopy. TmrAB is shown to have an unusual substrate specificity for diverse substrates including dyes, peptides and lipids.^[1] Nitroxide spin labels were attached at key positions in the transporter and allosteric coupling between NBDs and the TMDs as modulated by the nucleotides was monitored using PELDOR (DEER). Under physiological ATP concentration, the NBDs exist in a dynamic equilibrium of open and closed states, which is completely shifted to the later upon vanadate trapping. Despite the asymmetry of the ATP-ase sites, both the consensus and degenerate sites responds to nucleotides in a symmetric manner. The ATP-induced closure of the NBDs leads to a conformational allostery of the TMDs into an outward-facing conformation, similar to that observed with Sav1866 AMP-PNP structure. The conformational equilibrium of this allosteric regulation is further modulated by the substrate.

[1] A.Nöll, et al., Proc. Natl. Acad. Sci. USA **114**, (2017)

Posters

– 6. Protein structure to function –

P-145

Functional implications of the crystal structure of visual arrestin polar core mutant- R175ER. Batra-Safferling¹, J. Granzin¹, A. Stadler², A. Cousin¹, R. Schlesinger³¹Institute of Complex Systems, ICS-6: Structural Biochemistry, Forschungszentrum Jülich, Germany; ²Jülich Centre for Neutron Science (JCNS-1) & Institute for Complex Systems (ICS-1), Germany; ³Institut für Experimentalphysik, Freie Universität Berlin, Germany

In visual signaling, binding mechanism of arrestin requires photoactivation and phosphorylation of the GPCR rhodopsin, where the receptor bound phosphate groups cause displacement of the long C-tail 'activating' arrestin. Mutation of arginine 175 to glutamic acid (R175E), a central residue in the polar core and previously predicted 'phospho-sensor' leads to a pre-active arrestin that can terminate phototransduction by binding non-phosphorylated rhodopsin. Here, we present the crystal structure of R175E mutant arrestin that reveals significant differences compared to the basal state reported in full-length arrestin structures such as disruption of hydrogen bond network in the polar core, and three-element interaction including disordering of several residues in the receptor-binding finger loop and the C-terminus. Additionally, R175E structure shows a 7.5° rotation of the amino and carboxy-terminal domains relative to each other. Consistent to the biochemical data, our structure suggests an important role of R29 in the initial activation step of C-tail release. Comparison of the crystal structures of basal arrestin and R175E mutant provide insights into the mechanism of arrestin activation, where binding of the receptor likely induces structural changes mimicked as in R175E.

P-147

Enzyme allosteric effect without significant conformational changes: role of dynamics and entropyP. M. Bisch¹, D. Perahia², P. V. Renault de Barros³¹Universidade Federal do Rio de Janeiro (UFRJ), Brazil; ²Ecole Normale Supérieure de Cachan, France; ³Institut Biomolécules Max Mousseron (IBMM), Montpellier, France

Allostery is a process by which binding of a molecule to one site alters the activity of a protein at another site; it is a common and efficient strategy to control their activity. Classical models consider that allostery is associated to large conformational changes, but it occurs also in absence of significant rearrangements. The ligand-induced modulation of vibration modes of a protein could be at the origin of allosteric effect and entropy would be the main component of free energy variations. We adopt a theoretical approach based on vibrational normal mode analysis within a simple harmonic model to put forward the role of dynamics and entropic effects. We present an investigation on allostery in cysteine proteases from the Papain family, including cathepsins from Trypanosoma causatives of important tropical diseases, like Chagas disease and African sleeping sickness. Using a set of computational and theoretical methods, we investigate possible allosteric sites and the role of some ligands in the regulation of the activity of these cathepsins. The understanding about dynamic and allostery of these target could help the rational design of new drugs, particularly important perspective since allosteric drugs could be more specific, efficient and cause less side effects.

P-146

Mechanism of electron transfer in cytochrome C oxidase elucidated by protein microenvironment change

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Cytochrome C oxidase (also known as complex IV) is the last enzyme in the electron transport chain of aerobic cellular respiration, happening at mitochondria. This enzyme receives 4 electrons from 4 cytochrome C molecules and transfer those to the molecular oxygen, the terminal electron acceptor. These 4 electrons sequentially reduce the prosthetic groups of cytochrome C oxidase and produce 3 reduced intermediates. Finally, the molecular oxygen is reduced to water by accepting all the 4 electrons. Crystal structures of all the reduced intermediates of cytochrome C oxidase are available from *Bos taurus*. Here we have computed the protein microenvironments around amino acids from the crystal structures of cytochrome C oxidase intermediates. Sequential comparison of different forms of cytochrome C oxidase indicates a huge change in protein microenvironments as a consequence of single electron transfer in each step. Amino acids involved in protein microenvironment changes are most likely affected by the single electron transfer process. This protein microenvironment change could be used as an effective measure to understand the mechanism of electron transfer in complex IV.

P-148

When Bio meets Nano: an affordable BioSAXS solution for shared X-ray instrumentation facilities

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Small-angle X-ray scattering (SAXS) applied to protein solutions has become an accepted structural biology technique. *In situ* measurements provide information e.g. about protein size and shape, folding/unfolding, aggregation, stability and molecular weight.

BioSAXS beamlines at synchrotrons grant instrument time only for selected research topics. Their accessibility is limited and may not be available at a convenient time or for routine measurements. Single-purpose BioSAXS lab instrumentation offers an alternative, but is expensive and thus often not affordable.

With the *Empyrean Nano edition* we recently introduced a unique, versatile X-ray scattering platform that not only provides excellent BioSAXS data, but is more generally applicable for the analysis of structures and dimensions in a variety of (nano-)materials and soft matter (e.g. colloids, lipids, polymers, surfactants, nanopowders, nanocomposites, nanofilms). Combining several scattering and diffraction techniques and covering a wide range of scattering angles, the instrument is a cost-effective solution for shared X-ray instrumentation facilities to support a variety of research topics in bio- and nanosciences.

The concept of the instrument will be explained and its BioSAXS performance will be demonstrated.

Posters

– 6. Protein structure to function –

P-149

The interfacial self-assembly of BslA and its application in stabilising anisotropic emulsion dropsK. M. Bromley¹, R. J. Morris¹, M. Schor¹, N. R. Stanley-Wall², C. E. MacPhee¹¹Department of Physics, University of Edinburgh, UK;²School of Life Sciences, University of Dundee, UK

BslA is an interfacial protein produced by the Gram-positive bacterium *Bacillus subtilis*. Its interfacial activity is mediated by the presence of a large surface exposed hydrophobic patch, similar to surface-active proteins known as hydrophobins. I will demonstrate that although functionally similar, BslA's adsorption mechanism is distinct from hydrophobins. BslA remains monomeric in aqueous media as residues in the hydrophobic cap are shielded from surrounding water by adopting a random coil conformation. When BslA adsorbs onto an interface the hydrophobic cap twists outwards, refolding into a three-stranded β -sheet conformation. The adsorbed form is then able to self-assemble into a 2D rectangular lattice thus forming an interfacial elastic film. This limited structural metamorphosis represents a previously unidentified environmentally responsive mechanism for interfacial stabilization by proteins.

The interfacial function of BslA films can be exploited to stabilise industrially relevant multiphase formulations such as emulsions. I will show that BslA can arrest dynamic emulsion states, producing different droplet morphologies depending on emulsification conditions. Finally, I will show how BslA can be utilised to enable the design of droplets with a chosen shape and size.

P-151

Molecular details of RNA methyltransferase activity and allosteric regulation: A simulation studyJ. A. Bueren-Calabuig^{1,2}, M. Bage¹, V. H. Cowling¹, A. V. Pisiakov^{1,2}¹School of Life Sciences, University of Dundee, UK; ²School of Science and Engineering, University of Dundee, UK

The “cap” structure added to the 5' end of mRNA transcripts is critical for gene expression, being required for transcript processing and translation initiation. One of the key enzymes is RNA guanine-7 methyltransferase (RNMT). From biochemical studies it was known that activity of human RNMT is regulated by a small protein RAM. Through molecular dynamics (MD) and accelerated MD (AMD) simulations, performed using the first crystal structure of the human RNMT-RAM complex, we revealed the molecular mechanism of this allosteric regulation [1]: RAM stabilises the RNMT lobe region and helix A, which controls substrate binding pockets. We have also designed mutants that mimic the effect of RAM in stabilizing the lobe. Furthermore, we will present new results explaining the cooperative binding of ligands - GpppG cap and S-adenosyl methionine, a methyl donor - in the RNMT active site.

This work contributes to a better understanding of the mechanisms of regulated gene expression. In addition, a recent structure of Zika virus methyltransferase (ZIKV NS5 protein) [2] revealed high similarity to human RNMT, offering ways for structure-based rational design of antiviral inhibitors against Zika and other flaviviruses.

1) D. Varshney *et al.* *NAR*, **44** (2016) 10423.2) W. Duan *et al.* *EMBO J* (2017).

P-150

Is full cis-trans chromophore isomerization required for full biological activity of rhodopsins?K. Bryl¹, K. Yoshihara²¹University of Warmia and Mazury, Department of Physics and Biophysics, Poland; ²Suntory Institute for Bioorganic Research, SUNBOR, Retired, Japan

Light absorption by rhodopsin (RH), bacteriorhodopsin (bR), halorhodopsin (hR) triggers their conversion in which cis-trans isomerization of the chromophore is believed to be a primary step in this reaction. The problem whether full cis-trans isomerization is a prerequisite for full biological activity of rhodopsins is still open. The experiments with rhodopsins regenerated with retinal analogues: eight-, seven-, six-, five-membered ring retinal were performed. Flash photolysis and photoelectric data indicate that confinement of the chromophore double bond rotation limits rhodopsins photoreaction. Potentiometric and pH sensitive dyes show that even full blocking of double bonds rotation does not lead to termination the rhodopsins activity: bR pumps protons, hR chloride ions, RH generates small membrane potential. Experiments performed in model systems demonstrate that limited activity of rhodopsins is too low to activate ATPase or visual cascade. These results demonstrate that: an appearance of early intermediates is dependent on the flexibility of the chromophore, limited rhodopsins activity can be achieved with blocked trans-cis isomerization of the chromophore, and full chromophore cis-trans isomerization is required for full biological activity of rhodopsins.

P-152

Faster spinning probes and high proton content: when resolution meets sensitivity in solid-state NMR

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Solid-state NMR has the potential to be a major technique to study structure and dynamics of proteins. The need for large quantities of isotope labeled protein and the cumbersome analysis of moderately resolved spectra often restrains general applicability of this technique, and further improvements in sensitivity and resolution are crucial for routine protein investigations.

Here we show that these challenges can be overcome by employing ultra-fast magic-angle spinning (MAS) at rates larger than 100 kHz in small 0.7 mm rotors, at high magnetic fields. Under these conditions, efficient detection of ¹H nuclei becomes possible in fully-protonated systems. The use of 0.7 mm rotors reduces the sample requirements to less than 500 µg without sacrificing sensitivity. A drastic improvement in resolution and coherence lifetimes can be achieved, enabling the acquisition of multidimensional correlation experiments. This is demonstrated on samples of various molecular sizes in crystalline state, in native lipid environments and in amyloid fibrillar form. These systems provide a palette of targets to explore the size limits of 2D and 3D spectroscopy and develop approaches to obtain sequences specific information in proteins of large size or only available in sub-milligram quantities.

Posters

– 6. Protein structure to function –

P-153

Specific post-translational O-mycoloylations mediate protein targeting to the mycomembrane

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The outer membranes (OM) of members of the *Corynebacteriales* bacterial order, also called mycomembranes (MMs), harbor mycolic acids and unusual OM proteins (OMP). The signals that allow precursors of such proteins to be targeted to the MM remain uncharacterized. We report the molecular features responsible for OMP targeting to the MM of *Corynebacterium glutamicum*. We first investigated the partitioning of endogenous and recombinant PorA, PorH, PorB and PorC between bacterial compartments and demonstrated that they were both imported into the MM and secreted into the extracellular medium. A detailed investigation of cell extracts and purified proteins by top-down mass spectrometry, nuclear magnetic resonance spectroscopy and site-directed mutagenesis revealed specific and well-conserved post-translational modifications, including O-mycoloylation, pyroglutamylation and N-formylation, for MM-associated and secreted OMPs. Furthermore, we found that such modifications were essential for targeting to the mycomembrane and sufficient for OMP assembly into mycolic-acid containing lipid bilayers.

Reference : Carel C., Marcoux J., Réat V., Parra J., Latgé G., Laval F., Demange P., Burlet-Schiltz O., Milon A., Daffé M., Tropis M., Renault M. A. M. PNAS (2017), 114 (16) 4231–4236.

P-155

The structural basis of Vps75 histone chaperone tetramer

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Vps75 form a stable complex with the histone H3 acetyltransferase of Regulator of Ty1 Transposition 109 (Rtt109) to acetylate histone H3 lysines. Previous studies show that Vps75 adopts a homodimer. Our research indicates that the *penumocystis carinii* (Pc) protein Vps75 (PcVps75) forms a tetrameric structure. The X-ray crystal structure of PcVps75 tetramer was resolved at 2.1 Å resolution and revealed that its quaternary structure is calcium-dependent. Two homodimer of PcVps75 form a hamburger shape tetramer by covering histone binding area each other showing the structural evidence to the ‘self-chaperoning’ mechanism of histone chaperone.

P-154

Early stages of Parkin activation – a computational study

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Parkin is an E3-ubiquitin ligase involved in the regulation of mitophagy. Upon activation, it mediates the transfer of ubiquitin (Ub) from an ubiquitin-conjugating enzyme (E2) to specific substrate proteins, labelling those for degradation. Recent biochemical and structural data suggested that several factors are involved in Parkin activation. However, the available data cannot unambiguously explain the mutual effect of these factors or the detailed sequence of steps on the molecular scale.

In this work, we use a combination of computational tools to examine the effect of: i) phosphorylation of Parkin, ii) removal of the UBL domain, iii) allosteric regulation by phospho-ubiquitin (pUb), and iv) reported pathogenic mutations, on Parkin’s stability and activation mechanism. Our results suggest that i) phosphorylation alone is unlikely to promote the transition from the inactive to active conformation; ii) UBL removal might facilitate E2 binding, but its complete detachment from Parkin is rather improbable in a physiological context; iii) pUb binding stabilises the active conformation of Parkin likely priming the activation process; iv) mutations involving residues at the UBL/RING1 interface lead to structural rearrangements suggested to be involved in the activation mechanism.

P-156

***Chlamydia trachomatis* DsbA: A weakly oxidising oxidoreductase**

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Due to the rise in antibiotic drug resistance development of new antimicrobial drugs is crucial. DsbA proteins introduce disulfide bonds to proteins providing structural bracing for secreted virulence factors. Consequently DsbAs are potential targets for antimicrobial drugs.

Chlamydia trachomatis DsbA (CtDsbA) has oxidase activity and redox properties equivalent to other DsbAs with two significant differences; CtDsbA is the weakest oxidising DsbA studied and the active site disulfide is only mildly destabilising. The structure of CtDsbA is solved to 2.7 Å and reveals a typical DsbA fold. Unlike most other DsbAs the dipeptide separating the two active site cysteines of CtDsbA does not consist of a proline followed by an aromatic, polar or positively charged residue. Relative to other DsbAs, the two small uncharged residues in the CtDsbA dipeptide provides less stabilisation of the N-helix dipole of helix 1, which is the primary factor stabilising the nucleophilic thiolate in dithiol oxidoreductases. This is in agreement with our finding that CtDsbA is a weaker oxidase and has a mildly destabilising disulfide.

Characterisation of CtDsbA has broadened our understanding of the diversity observed among DsbA proteins and supports ongoing efforts to develop inhibitors for these proteins.

Posters

– 6. Protein structure to function –

P-157

Structure and function of drug efflux regulator proteins in *Acinetobacter baumannii*

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Multidrug efflux is a ubiquitous mechanism of drug resistance in pathogens. In *Acinetobacter baumannii*, eight efflux systems have been functionally characterised to date, each with the ability to respond to a wide array of molecules and environmental conditions. The expression of these efflux pumps is controlled by one or more transcriptional regulator proteins, from the TetR or LysR family. I am investigating four specific regulators in *A. baumannii* (AdeN, AdeL, AdeR, and AceR). These proteins contain two distinct domains: the DNA-binding domain (DBD) and the ligand-binding domain (LBD). Although the DBD has high sequence identity across family members, the LBD is markedly variable in order to accommodate the range of molecules taken up and exported by their constituent efflux pumps. My work explores the regulatory functions of each target protein, focusing on the binding capacities of likely partners. In my poster I will outline results of ligand screening and direct binding measurements (DSF, SPR, gel shift assays, and DNaseI footprinting).

P-159

Determining the molecular basis for interactions between the WW2 domain of WWP2 and target regions

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WWP2, an E3 ubiquitin ligase with a role in cancer, contains a Ca²⁺/phospholipid binding C2 domain, a HECT ligase catalytic domain and four WW domains consisting of ~35–40 amino acids each, able to recognise specific substrates containing PPXY motifs. WWP2 interacts with PTEN (tumour suppressor), Smads (part of the TGF- β signalling pathway, often deregulated in cancers), and the stem-cell specific transcription factor Oct4 (involved in cancer cell proliferation, dedifferentiation, and chemotherapy resistance) through the WW domains. Human cancer tissue samples present altered isoform expression patterns for WWP2 (Soond *et al.*, *Biochim. Biophys. Acta* **1832**, 2127). Inhibitory Smad7, involved in downregulating TGF- β signalling, interacts with WW4, but not with WW1 (Soond & Chantry, *Oncogene* **30**, 2451). Oct4 was seen to interact with WW2 (Xu *et al.*, *Cell Res.* **19**, 561). Using NMR spectroscopy, we show that the WW2 domain (expressed as a fusion protein with GB1 for solubility) is in conformational exchange when isolated, indicating it is not fully folded. A peptide derived from Smad7 binds WW2 and stabilises its structure. Complete backbone assignment of bound WW2 provides secondary structure information. A peptide from Oct4 causes a similar, but not identical, effect on the structure.

P-158

Protein functional prediction in genomic scale by structural modeling

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The functional prediction aims to solve a central challenge of the post-genomic era, to infer function for the product of all the genes of an organism. The traditional strategy is to search for sequences with known functions that have similarity to the target sequence. However, a lot of sequences still classified as hypothetical or unknown function. Even since it is well accepted that structures of proteins are close related to the genes functions, experimental determination of protein structure still be an expensive and difficult task. To overcome that theoretical and computational effort are in order. The first step for structural annotation of a gene is the construction of a 3D model for the coded protein by comparative modeling using templates from structure databases. Further functional information is recovered from structural evolutionary relationships and search for structural domains. We present here computational methods that provide means to exploit the concept of sequence-structure-function relationship in a high-throughput genomic scale, by using two main workflows; **MHOLline** which combines a specific set of programs for comparative modeling approach and **AS-AProt** that is a computational workflow for protein functional prediction using structural information.

P-160

Human SET/TAF-I β and plant NRP1 are similarly inhibited by cytochrome c in the cell nucleus

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Genome integrity is constantly battered by genotoxic agents. These can induce DNA damage that leads to cell death if not properly repaired. Most studies on the DNA repair process have focused on yeast and mammals, in which histone chaperones have been revealed as key regulators for DNA to be accessible to repair machinery. However, knowledge of their exact role in DNA damage response is far from complete, in particular in plants. Our recent studies reveal that the closely related histone chaperones human SET/TAF-I β and plant NRP1 similarly interact with cytochrome c in the cell nucleus upon DNA damage. Interestingly, cytochrome c competitively hinders the binding of SET/TAF-I β and NRP1 to core histones, thereby locking their histone binding domains and inhibiting their nucleosome assembly activities. The underlying molecular mechanism of nucleosome disassembly/reassembly needed for DNA repair is thus highly conserved throughout evolution [1, 2].

[1] González-Arzola K. *et al.* (2015) PNAS 112, 9908.

[2] González-Arzola K. *et al.* (2017) NAR 45, 2150.

Posters

– 6. Protein structure to function –

P-161

Characterization of a putative mannosidase selected in a metagenomic approach

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Metagenomic screening studies from diverse environments have been successful in isolating many novel enzymes that can degrade lignocellulosic biomass. The aim of this research was to express, purify and structurally characterize a novel enzyme. The most efficient cellulolytic colonies from metagenomic expression library of the *Capra hircus* were sequenced and screened for genes. The protein was cloned, expressed and purified by IMAC. DLS measurements showed a particle with a hydrodynamic diameter of 4.36 nm and an estimated MW of 105.6 ± 18.8 kDa, which indicates that the particles are present in an homodimeric form. Protein structure was modeled by homology modeling revealing a single-domain protein adopting a $(\alpha/\beta)_8$ barrel fold and the conserved catalytic active site of bacterial endo- α -mannosidase enzymes. The homology model presents 29.8, 14.5 and 55.9% of α -helices, β -sheets, and random coils, respectively, and is in agreement with the secondary structure determined through circular dichroism spectroscopy at pH 9.0. In addition, protein crystal screening experiments are also in progress. Characterization of this enzyme may lead to novel features in bacterial cell wall degradation mechanism.

P-163

Analysis of the 3D structure of dystrophin fragments in the presence of isotropic bicelles

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The protection of cell membrane against shearing stresses is ensured by cytoskeletal proteins. Among them, dystrophin, a large, amphipathic and fibrous protein, plays a key-role in muscle and its absence leads to Duchenne Muscular Dystrophy. Exhaustive knowledge of dystrophin membrane interaction is needed to help for gene therapy strategies. Previous work highlighted that the interfacial properties of dystrophin are modulated the nature of lipids, as well as the membrane curvature, that play key-roles in the physiology of the muscle cell. To understand what might be the role of these interactions *in vivo*, we aimed to determine the structure and the interaction mode of dystrophin fragments in the presence of zwitterionic or anionic isotropic bicelles. In this study, SAS data were coupled to molecular modeling in order to propose all-atom models of dystrophin fragments bound to membrane lipids. By using circular dichroism, Trp fluorescence and SANS analysis of protein/contrast-matched bicelles complexes, we show that binding to bicelles induces conformational changes of the dystrophin fragments. We describe the low resolution 3D structure modifications and we propose coarse-grained models of the complexes made of a filamentous protein bound to membrane mimics.

P-162

Structural basis of mitochondrial dysfunction in response to cytochrome c phosphorylation

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Regulation of mitochondrial activity allows cells to adapt to changing conditions and to control oxidative stress, and its dysfunction can lead to hypoxia-dependent pathologies. Although cytochrome *c* phosphorylation—in particular, at tyrosine 48—is a key modulator of mitochondrial signaling, its action and molecular basis remain unknown. Here, we mimic phosphorylation of cytochrome *c* by replacing tyrosine 48 with *p*-carboxy-methyl-L-phenylalanine (*p*CMF). The NMR structure of the resulting mutant reveals significant conformational shifts and enhanced dynamics around *p*CMF that could explain changes observed in its functionality: the mutation impairs cytochrome *c* diffusion between respiratory complexes, enhances hemeprotein peroxidase and ROS scavenging activities and hinders caspase-dependent apoptosis. Our findings provide a framework to further investigate the modulation of mitochondrial activity by phosphorylated cytochrome *c* and to develop novel therapeutic approaches based on its pro-survival effects (*PNAS Plus* 2017).

P-164

Mechanism of chromosome synapsis by SYCP1 revealed through X-ray crystallography, MALS and SAXS

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In meiosis, homologous chromosomes are tethered along their entire length by a zipper-like protein assembly - the synaptonemal complex (SC). This structure is essential for the formation of crossovers which bind homologues and introduce genetic diversity. SYCP1 underpins the SC architecture, self-assembling into a 100 nm wide lattice-like array between homologues. In this, SYCP1 C-termini are chromosomally bound whilst the N-termini act as the teeth of the zipper, self-associating at the SC midline.

We determined the structure of obligate unassembled SYCP1 through multi-angle light scattering and small-angle X-ray scattering experiments. We find that SYCP1 contains a 50 nm central helical domain consisting of an N-terminal four-helical bundle which splays into two dimeric coiled-coils.

Crystal structures and biophysical characterisation of the N- and C-terminal regions of SYCP1 that mediate self-assembly reveal two contrasting mechanisms that drive lattice-like assembly. Whilst C-termini undergo pH-dependent antiparallel associations, triggering interaction with DNA, N-termini cooperatively interact head-to-head with opposing filaments. Finally, we propose a molecular model for homologue tethering by SYCP1.

Posters

– 6. Protein structure to function –

P-165

Hofmeister ions induced changes in conformation and activity of 3C proteaseE. Dušeková¹, E. Sedlák²¹Department of Biophysics, University of Pavol Jozef Šafárik, Jesenná 5, 040 01, Košice, Slovakia; ²Centre for Interdisciplinary Biosciences, University of Pavol Jozef Šafárik, Jesenná 5, 040 01, Košice, Slovakia

Serine proteases such as Xa factor, enterokinase and thrombin have been used for many years to remove affinity and solubility tags from recombinant proteins but due to their non-specific cleavage of proteins they were replaced by viral proteases, such as 3C protease and TEV proteases. These cysteine proteases have stringent sequence specificity and enhanced activity compared to mentioned serine proteases. Based on previous works we decided to study, in detail, the effect of Hofmeister series of anions on properties such as catalytic activity, conformational stability, and how are related these properties in recombinant protein PV 3C protease. It has been shown that low *in vitro* catalytic activity of homologous HRV 3C protease can be increased by change of properties of solutions due to the Hofmeister effect. Our observations indicate that Hofmeister effect of ions has a potential to significantly modify catalytic properties of proteases both as regards affinity of the substrate and the enzyme catalytic rate.

This work was supported by grants VEGA 1/0423/16 and APVV-15-0069.

P-167

Low concentrations of GdnHCl have osmolyte-like effect on GGBP proteinA. V. Fonin, I. M. Kuznetsova, K. K. Turoverov
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The GdnHCl and urea-induced conformational changes of D-glucose/D-galactose-binding protein mutant GGBP/H152C labeled with fluorescent dye BADAN in apo and holoforms were investigated. Study of dye and tryptophan fluorescent characteristics allows analyzing GGBP/H152C structural dynamics. The different stability of N and C-terminal protein domains and different environment of BADAN linked to unfolded GGBP/H152C in GdnHCl and urea were shown. It was found that subdenaturational GdnHCl concentrations promote increase of glucose affinity to GGBP/H152C. The subdenaturational urea concentrations do not affect glucose binding affinity. Analogous results were observed by isothermal titration calorimetry when studying wild type protein interaction with glucose in same conditions. Considering GGBP molecules population as an ensemble of conformers in dynamic equilibrium we supposed that low GdnHCl concentrations cause an equilibrium shift toward conformers active centre of which are more complementary to ligand structure. This explains an increase of affinity of glucose to protein in such conditions. Obtained data allow to conclude that GdnHCl can be considered as concentration-dependent osmolyte-like agent. This work was supported by a grant RSCF 14-24-00131 and fellowship SP-1725.2015.4

P-166

Posttranslational modification and thermal stability of recombinant human serum albumin products

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Commercially available human serum albumin (HSA) products have been widely used in both laboratory and clinical fields. Posttranslational modification such as thiol oxidation and carbonylation, and the thermal stability of recombinant HSA (rHSA) products were examined. Products were obtained from Sigma-Aldrich Co. USA (products nos. A9731 (3 lots) expressed in *Oryza sativa*, A7223 (3 lots) expressed in *Pichia pastoris*, A6608 (3 lots) expressed in *Saccharomyces cerevisiae*). Thiol oxidation, carbonylation and the thermal stability were investigated by an HPLC (high-performance liquid chromatography) system, a protein carbonyl detection kit and a DSC (differential scanning calorimetry), respectively. Degree for both thiol oxidation and carbonylation of rHSA products was A9731 > A7223 > A6608. Values for *T_m* (midpoint temperature of denaturation process) of rHSA products were between 72–79 °C. Degree for the thermal stability monitored by *T_m* was A9731 > A7223 > A6608. This study shows that the bioprocess such as different expression systems of rHSA products results in the large differences in the chemical modification and the thermal stability in recombinant protein.

P-168

Orchestrated domain movement in catalysis by NADPH-cytochrome P450 reductaseS. Freeman^{1,2}, A. Martel², E. L. Raven¹, G. C. Roberts³¹Department of Chemistry, University of Leicester, UK; ²Institut Laue-Langevin, France; ³Department of Molecular & Cell Biology, University of Leicester, UK

NADPH-cytochrome P450 reductase (CPR) is a multi-domain redox enzyme which is a key component of the P450 mono-oxygenase drug-metabolizing system. Using small-angle X-ray scattering (SAXS) some evidence has been presented for a conformational equilibrium involving large-scale domain motions in this enzyme. We now study this proposed equilibrium using small-angle neutron scattering (SANS), under conditions where we are able to control the redox state of the enzyme precisely. We show that different redox states and buffer conditions have a profound effect on the conformational state of the enzyme. We present different ways to model the data based on multi-state systems. By altering the position of the conformational equilibrium by mutagenesis, we show that the presence of a greater proportion of the extended form leads to an enhanced ability to transfer electrons to cytochrome *c* on the millisecond timescale. Domain motion is thus intrinsically linked to the functionality of the enzyme, and we can define the position of the conformational equilibrium for individual steps in the catalytic cycle. We have also been able to describe for the first time the nature of the complex between CPR and a redox partner protein, cytochrome *c*, in solution by means of deuteration and contrast matching in SANS.

Posters

– 6. Protein structure to function –

P-169

Functional and thermodynamic characterization of ATP analogs binding P2X2 receptors

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P2X receptors (P2XRs) are ligand-gated ion channels that are activated by adenosine triphosphate (ATP). P2XRs are present in a wide variety of tissues and are involved in cardiovascular, gastrointestinal and respiratory diseases, among others. Therefore P2XRs have become important molecular targets for the pharmaceutical industry. However, there are still unresolved topics associated to their function such as 1) how different ligand analogues evoke different P2XRs activities and 2) how ligand structural differences change the energetic landscape of the ligand binding. The goal of this work is to study the relationship between these two aspects. We measured sodium currents evoked by γ -[2-azidoethyl]-ATP, EDA-ATP and ATP by using whole-cell patch clamp. In addition, to study the energetic landscape of the binding site, we performed dynamic force spectroscopy to speed up the rupture of receptor-ligand complex. Our results show that γ -[2-azidoethyl]-ATP is a partial agonist of P2X2R but does not desensitize the receptor, while EDA-ATP is a full agonist that produces desensitization. In terms of energetics, both EDA-ATP and γ -[2-azidoethyl]-ATP show similar activation energy barriers, but EDA-ATP's barrier width is narrower. Funded by ICM-P10-035F and DPI-CONICYT 20140080.

P-171

Protein and sodium pumping by pyrophosphatasesA. Goldman^{1,2}, C. Wilkinson¹, J. Kellosalo², T. Kajander², S. Harborne¹, N. Shah¹, A. Turku², S. Harris¹, K. Vidilaseris²¹University of Leeds, UK; ²University of Helsinki, Finland

Membrane-integral pyrophosphatases (mPPases) couple pumping of Na⁺ and/or H⁺ ions across a membrane to the hydrolysis of pyrophosphate (PP_i). They are potential drug targets as there are no human homologues, and they are important in the infectivity of protozoan parasites. Our structures of Na⁺-pumping *Thermotoga maritima* mPPase (TmPPase), in comparison with H⁺-pumping mung bean mPPase explain ion selectivity, and suggest how closure of the active site by helices 5–6 causes helix 12 to move down, springing a ‘molecular mousetrap’ that activates the water nucleophile and leads to ion pumping. Atomistic molecular dynamics simulations confirm that the 5-6 loop is mobile in the absence of substrate, and suggest that interactions of this loop with the substrate and its associated Mg²⁺ hold the loop closed. Finally, imidiphosphonate and methylene diphosphonate, non-hydrolysable analogues of PP_i with NH or CH₂ bridges, induce low levels of ion-pumping, while another non-hydrolyzable substrate analogue, etidronate, with a CHOH bridge, does not as the 5-6 loop can not close. Ion pumping must therefore precede substrate hydrolysis: that the mechanism of the enzyme is “binding change”, like the rotary ATPases, even though there is no other mechanistic or structural similarity.

P-170

The effect of dissociation and rebinding of FAD cofactor on the properties of glucose oxidaseK. Garajová¹, E. Dušeková², E. Sedlák³¹Department of Biochemistry, Institute of Chemistry, P.J. Šafárik University, Moyzesova 11, 040 01 Košice, Slovakia;²Department of Biophysics, Institute of Physics, P.J. Šafárik University, Jesenná 5, 040 01 Košice, Slovakia; ³Centre for Interdisciplinary Biosciences, P.J. Šafárik University, Jesenná 5, 040 01 Košice, Slovakia

The role of glucose oxidase (GOX) as a part of glucose biosensors used in medicine brought our interest in this protein. We focused on the changes in the structure of GOX and in its properties upon the FAD dissociation from the protein. The reversibility of this process was determined from the renewal of structure as well as studied properties. The dissociation of FAD was achieved using low pH in combination with high ammonium sulphate concentration, while cofactor rebinding to molecule was done by means of incubation of apo-GOX with external source of FAD. We used absorbance, fluorescence and circular dichroism to describe structure changes, while SDS-PAGE, size exclusion chromatography and glutaraldehyde cross-linking allowed us to determine oligomeric state. Upon deffavination protein acquires the structure of molten-globule state with lower stability. Upon reffavination the native-like structure was observed with 65% of its native activity. We can conclude, that apo-GOX remains in its dimeric form as molten-globule with the ability to refold to native-like structure.

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P-172

Annexin-phospholipid interactions mediating membrane linkageD. Grill¹, W. de Vries², A. L. Matos¹, M. Pejic¹, H.-J. Galla³, B. J. Ravoo², V. Gerke¹¹Institute of Medical Biochemistry, ZMBE, University of Münster, Germany; ²Institute of Organic Chemistry, University of Münster, Germany; ³Institute of Biochemistry, University of Münster, Germany

The dynamic organization of cellular membranes into spatially and temporally organized domains is relevant to many signal transduction and membrane trafficking events. This organization is driven by intrinsic properties of membrane lipids as well as integral and peripherally associated membrane proteins. Of importance, associated proteins can specifically interact with certain membrane lipids and affect their distribution. The annexins are a family of such membrane-binding proteins that can induce the formation of cholesterol and phosphatidylinositol-4,5-bisphosphate rich membrane microdomains in artificial and cellular membrane systems. In addition some annexins have been proposed to crosslink membrane surface although the molecular basis of this linkage is not known, to address this we performed a quantitative analysis of the thermodynamic state of annexin A2 (AnxA2) bound to artificial membrane systems using different genetic and chemically modified AnxA2 derivatives. We employed the Quartz Crystal Microbalance with Dissipation technique in combination with Optical Density and Dynamic Light Scattering measurements to address the fundamental question whether monomeric AnxA2 is capable of membrane crosslinking or AnxA2-dimerization is required.

Posters

– 6. Protein structure to function –

P-173

Phosphorylation of cytochrome c: Structure, dynamics and functions

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Phosphorylation cytochrome *c* (*Cc*) at tyrosine 48 relates to a variety of human diseases, but analyzing this modification is hampered by low purification yields (Guerra-Castellano *et al.*, *Chem. Eur. J.* 2015; *BBA* 2016). Hence we resorted to make a close mimic of phosphorylated *Cc*, by optimizing the synthesis of the non-canonical amino acid *p*-carboxymethyl-L-phenylalanine (*p*CMF) and its site-specific incorporation. *Cc* has a globular structure with four α -helices and a heme group, which shows two axial ligands. The loss of the Met80 as an axial ligand upon pH increment is called alkaline transition. We analyzed the pK_a value of the mutant by visible spectroscopy, NMR, EPR and Raman spectroscopy; and concluded that the alkaline transition becomes physiologically relevant upon *Cc* phosphorylation (Guerra-Castellano *et al.*, *Chem. Eur. J.* 2015).

NMR relaxation data shows enhanced dynamics of the regions surrounding the Y48*p*CMF mutation. We further determined the solution 3D structure, very similar to that of the WT species (Guerra-Castellano *et al.*, *PNAS* 2017).

Such enhanced dynamics and subtle structural changes affect the activity of *Cc* and the binding mode to its partners, with strong implications on cell metabolism and signaling (Guerra-Castellano *et al.*, *PNAS* 2017).

P-175

Revisiting the functional classification of class A β -lactamases

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Antimicrobial resistance is one of the main threats to public health in the 21st century, as recognized by the WHO. Both the use of common and last resort antibiotics, particularly β -lactamic compounds, is seriously compromised because of the continuous proliferation of resistant bacterial strains and proteins such as β -lactamases.

While many genes and structures of this protein family have been identified, much of this information has yet to be systematically ordered and there is not a unified method of cataloguing these proteins based on their activity, partly because antibiotic screening has not always been consistent. The most used classification scheme was proposed by Bush and Jacoby (1995), who separated enzymes by their substrate and inhibitor profiles. However, even this scheme presents problems, as it requires time-consuming experiments to classify a new protein. Moreover, this classification appears not to be exhaustive and it has been suggested that new functional classes should be defined.

We have analyzed the sequence, structure and local energetics of the functional classes corresponding to Class A β -lactamases. By identifying and comparing their functional determinants, we detected new relationships between structure, conformational dynamics and catalysis. Moreover, this work contributes to the development of a method to automatically and consistently classify these enzymes according to their activity.

P-174

Unravelling the intrinsic dynamics of cyclophilinA during protein-ligand interaction

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CyclophilinA (CypA) is a member of family of cyclophilin proteins known to catalyze the *cis-trans* interconversion of proline peptide bonds. Role of CypA in various disease conditions such as cancer, inflammatory disorders and viral infections is well documented. CyclosporinA (CsA), a nanomolar affinity immunosuppressant drug used to prevent organ transplant rejection in humans mediates its biological activity by targeting cyclophilins. Hence, there has been profound interest in designing specific non-peptidic cyclophilin inhibitors with non-immunosuppressive side effects mediated through various human cyclophilin isoforms. Previous characterization of distinct conformational sub-states in CypA using solution NMR spectroscopy implies role of conformational sampling mechanism. ¹⁵N NMR backbone relaxation experiments were employed to characterize the influence of slower time scale motions within single domain CypA protein during ligand binding. Understanding how the equilibrium is altered upon ligand binding through analysis of NMR based chemical shift changes will reveal role of coupled network of residues. Dissecting intrinsic protein dynamics offers a tantalising prospect of a ‘conformational trapping’ strategy useful for the development of isoform selective ligands.

P-176

On the origin and variation of colours in lobster carapace

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In protein structure and function an archetypal case of coloration is of lobster shell, known from its colour change on cooking, and which derives from a 16-mer of molecular weight 320 kDa of astaxanthins and proteins, ‘crustacyanin’. The molecular colour-tuning parameters of the 100nm bathochromic shift of the two astaxanthins in the β -crustacyanin protein dimer (40 kDa) portion, versus free astaxanthin, are known from our X-ray crystal structure, and whose crystals possess a distinct blue color. By analyzing the response of UV-vis spectra of a model of astaxanthin to increases in pH, we find that enolate formation, possible within the protein environment, is associated with a large bathochromic shift. The ultrastructure of the full crustacyanin, responsible for the full bathochromic shift of 150nm, has been studied using EM, SAXS and molecular modelling.

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Determining membrane bound protein structures by infrared reflection-absorption spectroscopy

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The structure of membrane bound proteins is difficult to obtain, yet in many cases, it is the active conformation. We used infrared reflection-absorption spectroscopy to determine a major conformational change in the membrane reshaping EH-domain-containing protein 2 (EHD2). EHD2 is a dynamin-related ATPase that confines caveolae to the cell surface. For this, EHD2 first binds to the membrane, then oligomerizes, and finally detaches, in a stringently regulated mechanistic cycle. We demonstrate that EHD2 adopts an open conformation upon membrane binding. We show that ATP binding enables partial insertion of EHD2 into the membrane, where oligomerization occurs. ATP hydrolysis is related to detachment of EHD2 from the membrane. This stringently regulated mechanistic cycle might be prototypical for a large family of proteins involved in membrane fission and may open avenues to control the process in vivo.

P-179

Resonance raman spectroscopy provides insights into biological hydrogen conversion

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Catalysing H₂ cycling, hydrogenases (H₂ases) are model metalloenzymes for sustainable energy conversion approaches. We introduce resonance Raman (RR) spectroscopy as a powerful tool to selectively probe their catalytic intermediates. O₂-tolerant [NiFe] H₂ases catalyse H₂ cleavage at industrially relevant O₂ levels. Using an experimental and theoretical approach, their resting state was shown to exhibit a seesaw-shaped Ni geometry and an *S* = 0 electronic ground state, as proposed to be mandatory for H₂ binding. Likewise, a second key intermediate was demonstrated to be formed *via* H⁺-transfer from the substrate binding site towards a Ni-bound cysteine. Based on tailored biomimetic compounds, we also identified RR markers of active site sulphenates essential for their O₂ tolerance.

[FeFe] hydrogenases are superior H₂ evolution catalysts. Exploring their [FeFe] and [4Fe4S] sub-centres in both native and non-native variants we identified a novel transient species with an unusual electronic ground state as the primary catalytic intermediate, thereby providing a key to the understanding of biological H₂ evolution.

In a wider sense, these studies highlight the capability of RR spectroscopy to selectively explore properties and intermediates of complex metalloenzymes in detail.

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Dynamic changes in complement component 3 in the presence of thrombomodulin's lectin-like domain

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Complement component 3 (C3) is at the junction of three different complement activation pathways (classical, lectin, and alternative). Activation to C3b is a key step in the innate immune response that allows for the formation of important multi-protein complexes that ultimately participate in pathogen clearance. When misregulated, however, complement can lead to inflammatory disease and autoimmune disorders. Several regulatory proteins for C3b are known, but the molecular details of interactions between these proteins have not yet been elucidated. Thrombomodulin (TM), and specifically its N-terminal lectin-like domain (TMD1), has been identified as a possible regulator of complement through interactions with C3 or C3b. We have used fluorescence-based assays and hydrogen/deuterium exchange mass spectrometry (HDXMS) to study the interaction of TMD1 with C3 and C3b. We have found that TMD1 interacts with C3b, and there is a lesser interaction with C3. TMD1 tends to make C3b more accessible to deuterium exchange, while C3 tends to be less accessible to deuterium exchange in the presence of TMD1. This difference suggests a possible role for TMD1 in regulating a key step along the complement pathway.

P-180

Structural transitions and enzymatic function: Case studies on superoxide reductase

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Superoxide, O₂^{•−}, is a reactive oxygen species (ROS) that damages biological metal sites of medical or industrial relevance. Catalysing O₂^{•−} reduction, superoxide reductases (SOR) represent model enzymes for ROS detoxification.

IR difference spectroscopy and DFT calculations revealed the reversible dissociation of an iron-bound glutamate during reductive activation of a 1Fe-SOR. Concomitant changes of other amino acids and the protein backbone suggest a global structural transition and cooperative activation. Likewise, normal mode and essential dynamics analyses of a 2Fe-SOR identified domain movements that may allow conformational proofreading for cellular electron donors. These collective modes of motion also enable molecular configurations with decreased tunnelling distances between the enzyme's iron centres. However, pathways analyses and MD simulations demonstrate that electron transfer is unaffected, suggesting that the unperturbed structure is optimized for this process. Consistently, the fastest tunnelling route is accessible *via* the equilibrium geometry, and an included *quasi* conserved tyrosine may enable hopping.

In a wider sense, these studies demonstrate the functional relevance of structural dynamics, and strategies for its analysis are provided.

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Structural basis for dimerization and RNA binding of avian infectious bronchitis virus nsp9

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Infectious bronchitis virus (IBV), a representative gamma-CoV which causes significant losses in the global poultry industry. IBV encodes two large polyproteins, which are proteolytically processed into 15–16 nonstructural proteins (nsps) that make significant contributions to viral replication and transcription. Among them, nsp9 plays an essential role in viral replication by forming a homodimer that binds single-stranded RNA. Disrupting nsp9 dimerization is a potential anti-CoV therapy. Here we determined the crystal structure of IBV nsp9 at 2.5 Å. IBV nsp9 forms a homodimer via interactions across a hydrophobic interface, which consists of two parallel alpha helices. The IBV nsp9 dimer resembles that of SARS-CoV nsp9, indicating that this type of dimerization is conserved among all CoV. This makes disruption of the dimeric interface an excellent strategy for developing anti-CoV therapies. To facilitate this effort, we characterized the roles of six conserved residues on this interface using site-directed mutagenesis and a multitude of biochemical and biophysical methods. We found that three residues are critical for nsp9 dimerization and its ability to bind RNA.

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Engineering and molecular dynamics simulations of calcium binding in the IgE receptor CD23

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Immunoglobulin E (IgE) is fundamental to the allergic response and its synthesis is regulated by binding to FcεRII/CD23, an unusual antibody receptor, being a calcium dependent (C-type) lectin that has lost its carbohydrate binding capability. Ca²⁺ binds to and increases CD23's affinity for IgE, and one of two Ca²⁺ binding sites usually present in C-type lectins is absent in human but present in mouse CD23. To understand if the loss of the second Ca²⁺ binding site has led to a regulatory gain/loss of function in human CD23, a panel of CD23 mutant proteins with increasingly 'mouse-like' sequences was generated. NMR verified the insertion of the second Ca²⁺ binding site while molecular dynamic simulations provided a means of understanding the flexibility of the proteins and revealed that binding of two Ca²⁺ tethers the derCD23 loops into position, limiting possible conformations for IgE binding. Complementary Biacore experiments indicated that higher calcium binding affinity may have come at a cost of weakened IgE binding; as data in the presence and absence of Ca²⁺ showed decreased binding affinities of the proteins for human IgE. This regulatory difference between mouse and human derCD23 could inform the development of CD23/IgE inhibitor therapeutics for the treatment of allergy.

P-182

Functional conversion from peptidyl-prolyl isomerase to protease by a single amino acid substitution

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Enzymatic function generally consists of three steps, substrate binding, catalytic reaction and product release. The catalytic reaction is often tightly linked to a specific motif like a catalytic triad of serine proteases. Thus, only a few changes of amino acid residues in the motif can change the enzymatic function. A peptidyl-prolyl isomerase, Pin1, catalyzes isomerization of pSer/Thr-Pro bond. Its activity is related to various cellular functions including suppression of Alzheimer's disease. Recently, we find that some mutants of Pin1 showed limited auto-proteolysis. We determined the target sequence by mass spectrometric analysis. We also investigated their proteolysis activities by physicochemical analysis and further mutational approaches.

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Anti-aggregation effect of thymoquinone and copper nano-particles: A biophysical insight

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Present study is based on the thermal aggregation and inhibition behaviour of serum albumin (SA) induced by rosin modified cationic surfactants. We have investigated the factors which are responsible for structural, conformational, morphological alteration in protein-structure which immense the aggregation of protein, and its prevention by using copper nano-particles (Cu-NPs) and thymoquinone (TQ). We are using non-ionic and cationic rosin modified surfactants but only cationic surfactant induced the conformational changes, binding of these surfactants are confirm by ITC experiments. Aggregation of SA is depend on the hydrophobicity, charge and temperature which are supported by our dye binding assays and ITC results. We have also performed the whole experiment at elevated temperature, higher concentration, pH below and above two units of pI of the protein. Nature of aggregates of SA was characterized by Th T and ANS dye binding assay, ITC experiments and electron microscopic studies. Cu-NPs and TQ are used to inhibit the aggregation, which binds with SA at particular binding site that induced the conformational changes and stabilize the overall conformation of protein. This inhibition process is drastically inhibited when TQ and Cu-NPs present together.

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– 6. Protein structure to function –

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Exploring structural characteristics of chitin deacetylases and chitin oligosaccharide deacetylasesA. Isvoran^{1,2}, M. Roman^{1,2}, D. L. Roman^{1,2}, A. Ciorsac³, V. Ostafe^{1,2}

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Chitin deacetylases (CD) and chitin oligosaccharide deacetylases (COD) are enzymes performing deacetylation of chitin, the second most abundant structural biopolymer found in nature. CD and COD play important roles in biotechnological applications and agriculture. Three-dimensional structures are already solved for members of the CD and COD families. However, there is still deficient knowledge about their substrate specificity and catalytic properties. Considering the importance of these enzymes, the exploration of their structural properties and conformational flexibility essentially contributes to the understanding of the molecular interactions they develop. In our study we consider the 11 structures of CD and COD present in the Protein Data Bank and analyze the structural properties (surface cavities, surface roughness) and explore the conformational flexibility of the entire structure and of particular regions and amino acids known to be involved in the interaction with ions and ligands.

P-187

The structure of the proton:fumarate symporter SLC26Dg in membranes probed with EPR spectroscopyE. A. Jaumann¹, Y.-N. Chang², T. F. Prisner¹, E. R. Geertsma², B. Joseph¹

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The members of the SLC26 family are multifunctional secondary anion transporters, whose dysfunctions in humans can lead to various diseases. So far, there is only one SLC26 crystal structure determined for a prokaryotic homolog SLC26Dg.[1] The transmembrane domain of the proton-coupled fumarate symporter SLC26Dg is connected to a cytosolic STAS domain. In the crystal structure, the hydrophilic STAS domain is located at an unnatural position that would place it in the lipid bilayer. We used site-directed spin labeling combined with pulsed electron-electron double resonance (PELDOR/DEER) distance measurements to investigate the structure and function of SLC26Dg. In this respect, spin labeling of cysteine mutants was optimized and PELDOR experiments were performed in detergent micelles and proteoliposomes. Our results revealed the dimer formation in membranes, highlighting the modulation of protein-protein interaction by the lipids. The PELDOR derived distances are used as constraints for rigid body docking to determine the dimer structure. Further, the orientation of the STAS domain in respect to the core domain in proteoliposomes is determined through multilateration.

[1] E.R. Geertsma et al., Nat. Struct. Mol. Biol. **22**, 2015

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PDB2CD: A novel tool for protein structure/function analysis

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Circular dichroism (CD) spectra display characteristic features of proteins attributable primarily to their secondary structure components, but also due to their topology. Because proteins within the same functional family are often structurally homologous even if their sequences are more distant, this homology in turn can lead to their CD spectra being similar as well.

The atomic structure of a protein (reflected in its PDB coordinates) can often provide a key factor for the identification of the function of a protein. However when such a structure is not available, the protein's CD spectrum can provide useful information as to its structure, and possibly function. We (Mavrides and Janes, 2017) have created a new online computational biophysics tool, PDB2CD, (<http://pdb2cd.cryst.bbk.ac.uk/>) which generates CD spectra from protein PDB coordinates. It can be used to generate the spectrum of the functional homologue of a known protein, enabling comparison with an experimentally-determined spectrum of that protein, hence providing valuable information on potential features in the protein's structure and, possibly, function.

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SNX16 regulates E-cadherin recycling through the mechanism of coordinated membrane and cargo bindingX. Jinxin¹, Z. Leilei², W. Chanjuan³, W. Junfeng³, P. Duanqing², S. Xiaodong², L. Jinsong¹

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E-cadherin is a major component of adherens junctions and the homeostasis of cell surface, and plays vital roles in cancer progression and induced somatic reprogramming. E-cadherin is dynamically regulated by endocytosis and recycling. We report here that SNX16 regulates the trafficking of E-cadherin. Inhibition of SNX16 in epithelial cells reduces cell surface E-cadherin and induces epithelial-mesenchymal transition. Mechanistically, SNX16 binds to the C-terminal cytoplasmic domain of E-cadherin and promotes the cell surface recycling of E-cadherin through the Rab11a mediated recycling endosome pathway. Structurally, SNX16 is a unique member of the SNX family proteins in that it contains a Coiled coil (CC) domain just downstream of PX domain. We solved the crystal structure of PX-CC unit of SNX16 and it reveals a shear shaped homodimer consisting of a parallel coiled coil and dyad-symmetric PX dimer. Based on the structure and biochemical studies, we identified a unique PI3P binding pocket in SNX16 that consists of both the PX and the CC domain and further propose a multivalent membrane binding model for SNX16. We further showed that the PPII/ α 2 loop, which is generally regarded as membrane insertion loop in PX family proteins, is involved in the E-cadherin binding in SNX16.

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– 6. Protein structure to function –

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Structural insights into the versatility of lipid binding by *Necator americanus* FAR-1 protein

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Fatty acid and retinol binding proteins (FARs) are a class of ~20 kDa lipid binding proteins abundantly expressed in nematodes including parasites that infect more than 400 million people globally. FARs are known to bind a wide range of ligands, and several secreted FARs were proposed to play a role in the parasite-host interactions by facilitating nutrient uptake from the host or by sequestering lipid mediators of inflammation in the host tissues.

The structure of the human hookworm *Necator americanus* FAR-1 (Na-FAR-1) has been determined, revealing a wedge-like fold containing a large internal cavity that accommodates lipid ligands. However, the molecular details of ligand binding by Na-FAR-1 remain unclear.

We have co-crystallised Na-FAR-1 with oleic acid, a natural fatty acid ligand, and determined the high-resolution structure of the complex, revealing several ligand binding sites in the Na-FAR-1 internal cavity that appear to have different affinities for the ligand. In addition, we are in the process of determining the solution structure of Na-FAR-1 in complex with phosphatidic acid, a phospholipid that interacts with Na-FAR-1 via a distinct mechanism. Our data have provided a clear insight into the structural basis of ligand recognition by Na-FAR-1.

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Structure based design of anti-thyroid drug using mammalian heme peroxidases as drug targets

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The mammalian heme peroxidase superfamily comprises four enzymes, myeloperoxidase (MPO), lactoperoxidase (LPO), eosinophil peroxidase (EPO) and thyroid peroxidase (TPO). These enzymes catalyze the hydrogen peroxide mediated oxidative reactions of inorganic substrates such as Cl^- , Br^- , I^- and SCN^- , organic aromatic compounds including phenols, catecholamines and catechols and experimental model compounds like aromatic amines and polychlorinated biphenyls. The structures of MPO and LPO revealed that the substrate binding sites are very similar in these enzymes. The structures have revealed that their substrate binding sites are located on the distal heme side. Currently used antithyroid drugs in clinical practice act by inhibiting the function of TPO. These drugs inhibit the catalytic action of LPO with similar IC_{50} values. To establish the modes of binding of compounds with substrate-like action as well as with inhibitory actions, we determined the structures of several enzyme-substrate and enzyme-inhibitor complexes. The LPO structures with antithyroid drugs and other model compounds have been determined. These studies have clearly indicated the exact modes of bindings of various compounds and suggested further modifications in the existing compounds for improving their potencies.

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Structure of CaBP6 from *E. histolytica* and its involvement in trophozoite proliferationV. R. C. Kandala¹, D. Verma¹, A. Murmu², G. Samudrala², A. Bhattacharya²¹Tata Institute of Fundamental Research, Mumbai, India;²Jawaharlal Nehru University, New Delhi, India

Cell cycle of *E. histolytica*, the etiological agent of amoebiasis, follows a novel pathway, which includes nuclear division without the nuclear membrane disassembly. We report a nuclear localized Ca^{2+} -binding protein from *E. histolytica* (EhCaBP6), which is associated with microtubules. We determined the 3D solution NMR structure of EhCaBP6. It forms a two-domain structure similar to Calmodulin. Down-regulation of EhCaBP6 affects cell proliferation by causing delays in transition from G1 to S phase, and inhibition of DNA synthesis and cytokinesis. EhCaBP6 affects cytokinesis by affecting microtubule dynamics and its knockdown results in multinucleated cells due to failed cytokinesis. We demonstrate that the localization of this protein in the nucleus is dependent on its ability to bind Ca^{2+} . We also demonstrate that EhCaBP6 modulates microtubule dynamics by increasing the rate of tubulin polymerization. Our results, including structural inferences, thus characterize EhCaBP6 as an unusual CaBP that, as a functional equivalent of Calmodulin in *E. histolytica*, is involved in regulating cell proliferation.

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Rotamer Libraries of spin and fluorescence labels aid structural interpretation of experimental dataD. Klose¹, S. Kucher², H.-J. Steinhoff², G. Jeschke¹¹Laboratory of Physical Chemistry, ETH Zurich, Switzerland; ²Department of Physics, University of Osnabrueck, Germany

In structural biology, the conformation of biomolecular complexes can be investigated by techniques that require covalent modification of protein side chains or nucleotides with labels, such as spin or fluorescence labels. The interpretation of such experimental data generally benefits from taking the label's spatial distribution into account. We previously established rotamer libraries for nitroxide spin labels, such as MTSSL, as a rapid approach that yielded good agreement with experimental data with only 0.2 nm standard deviation. Here, we extended this approach to significantly larger labels comprising up to eleven dihedral angles as found in fluorescence labels as well as in recently developed spin labels. We applied Monte Carlo sampling to efficiently generate large ensembles of label conformations, followed by clustering into a significantly smaller, yet representative, set of conformations, a rotamer library. These pre-computed libraries can be used to calculate the spatial distribution of a label within seconds on a desktop computer and thereby facilitate the comparison of experimental data to atomic structures, even for screening large ensembles or for refinement of structural models.

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Dynamics of membrane regulator GABARAP revealed by NMR and fluorescence combined with simulationsJ. Kubiak¹, C. Möller², T.-O. Peulen¹, P. Neudecker², C. A. M. Seidel¹¹Institut für molekulare physikalische Chemie, Heinrich-Heine-Universität Düsseldorf, Germany; ²ICS-6 (Structural Biochemistry), Forschungszentrum Jülich, Germany

The γ -aminobutyrate type A receptor-associated protein (GABARAP) from *H. sapiens* belongs to the Atg8 family of ubiquitin-like modifier proteins, involved in multiple cellular processes like membrane trafficking and fusion events (*FEBS J* 2009,276,p4989). The tertiary structure and dynamics is highly conserved in GABARAP-like proteins and hence these conserved dynamics likely play an important functional role. Using fluorescence spectroscopy (MFD FRET (*Methods in Enzymology* 2010,475,p455)) we found that static X-ray crystallography and NMR derived models does not describe important structural features of GABARAP in solution. We found functionally relevant multi-timescale conformational dynamics of the N-terminal helical domain of GABARAP, which we modelled with experimentally biased simulations. We show how hybrid use of fluorescence spectroscopy, NMR and other techniques aids a conformationally dynamic description of protein function.

P-195

SEIRA spectroscopic characterization of an oxygen-tolerant NAD+-reducing [NiFe] hydrogenase

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Due to its outstanding oxygen tolerance, the NAD(H)-linked soluble [NiFe] hydrogenase from *R. eutropha* (SH) is a promising candidate for biotechnological applications. SH and its separate diaphorase moiety (DD) were probed with an integrated approach of surface-enhanced IR absorption spectroscopy and electrochemistry to unravel application-relevant mechanistic and structural properties. SH and DD were bound to positively and negatively charged self-assembled monolayers (SAMs) on surface-sensitive Au electrodes. Pronounced amide absorptions of the protein backbone confirm the successful immobilization of both enzyme species on either SAM. A detailed kinetic study revealed a biphasic enzyme adsorption and an accompanying SAM-dependent reorientation for the two proteins. Immobilized SH and DD exhibit similar signatures in the amide I and II spectral region, suggesting the binding of SH *via* the diaphorase moiety. Minor differences between the two enzymes may be ascribed to structural differences in the separate DD module due to the lack of the stabilizing hydrogenase moiety. Ongoing potential-dependent measurements in the presence and absence of NADH also promise to provide insights into structural rearrangements of the SAM, the enzymes, and the nucleotide substrate.

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PAMs modulate molecular dynamics of nAChR alpha 7: direct observation by DXT and electrophysiologyT. Kubo¹, T. Baba^{1,2}, S. Ohashi¹, H. Inagaki¹, K. Ikezaki^{1,2}, K. Mio¹, H. Sekiguchi³, Y. C. Sasaki^{1,2}¹molprof & Operando-OIL, Natl Inst Advanced Industr Sci & Tech (AIST), Japan; ²Grad Schl of Frontier Sci, The Univ Tokyo, Japan; ³Japan Synch Rad Res Inst, SPring-8, Japan

The alpha 7 nicotinic acetylcholine receptor ($\alpha 7$ nAChR) is a ligand-gated ion channel; consisting of extracellular ligand-binding domains, intracellular loops, and a channel pore lined by homo-pentameric subunits. A characteristic feature of the receptor channel is that the ligand acetylcholine (ACh) induces the channel opening very quickly, and then it steeply transits to the “desensitization” state. To investigate single molecular dynamics of $\alpha 7$ nAChR in each state and the state-to-state transitions, we adopted the Diffracted X-ray Tracking (DXT) method, in which the X-ray diffraction spots from the gold nanocrystal labeled on an individual single protein are tracked and analyzed in real time (resolution ~ 100 μ s) and in highly accurate real space (\sim pm). Physiological functions of the channel were monitored by electrophysiological measurements. When a Positive Allosteric Modulator (PAM) Ivermectin (type I) or PNU-120596 (type II) alone was applied to $\alpha 7$ nAChR, both tilting and twisting motions were observed. Despite such molecular motions, channel pores were kept closed. When ACh was applied to the $\alpha 7$ pre-incubated with Ivermectin, significant potentiation both in the DXT dynamics and in inward currents was observed.

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A lectin from hyacinth plant bulbs having potent inhibitory activity against human cancer cells

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A monocote mannose specific lectin has been purified and characterised from Hyacinth bulbs. The agglutination activity of purified lectin was inhibited by monosaccharides D-mannose and glycoprotein (ovalbumin). Glycoprotein nature of purified lectin was confirmed by Periodic Acid Schiff's stain (PAS) whereas hydrodynamic studies by Dynamic light scattering (DLS) measurement showed that purified lectin is monomeric in nature with the molecular size of 2.38 ± 0.03 nm. Hyacinth lectin showed moderate pH and thermal stability, it retained hemagglutination activity in the range of pH from 6 to 8 and temperature up to 60°C. Hyacinth lectin also conquered the growth of human colon cancer cells (Caco-2) and cervical cancer cells (HeLa) with IC50 values of 127 μ g/ml and 158 μ g/ml, respectively, after 24 hour treatment with purified lectin. Morphological studies of treated cells (Caco-2 and HeLa) with hyacinth lectin by AO/EB staining indicated that purified lectin capable to induce apoptosis.

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– 6. Protein structure to function –

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Exploring a novel oligomerization mechanism of thermostable direct hemolysin

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Thermostable direct hemolysin (TDH) is a pore-forming toxin and a major virulence factor of pathogen *Vibrio parahaemolyticus*. It permeabilizes target host cells by generating pores on the cell membranes. Unlike other pore-forming toxins, TDH follows a distinct mode of action by forming oligomers in solution, without any prior membrane binding event. However, the reason behind this unique in-solution oligomerization propensity of TDH still remains unknown. In the present study we show the role of the C-terminal region of TDH in solution oligomerization by dissecting its interaction at protomer-protomer interface. Our study also highlights the mechanism by which a single conserved disulphide bond within the C-terminal region regulates the formation of the in-solution oligomers by TDH. Our study for the first time attempts to establish the possible mechanistic basis behind the unique tendency of TDH to form active oligomers without prior binding to the host cell membranes. Present work also opens new avenues to address the questions like why in-solution oligomerization is absent in the structurally-related eukaryotic pore-forming toxins.

P-198

Analysis of the nuclear protein structure

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The function of the protein is determined by the protein structure, highly conservative domain and motif play an important role in performing function. In this paper, the structure and function of 1505 proteins which can be classified into 6 subnuclear locations were analyzed. The dataset is constructed based on the SwissProt database. The domains and functions of subnuclear proteins are searched and the secondary structure and tertiary structure diagram of the domains can be found in the PDB database. The characteristic information can be used to understanding the structure and function of nucleoprotein.

Key words: cell nucleus; protein; domain; secondary structure; tertiary structure

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Structural determinants of the MAP6D1 peptides interaction with tubulin and calmodulin

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We investigate the structure and microtubule stabilization of two peptides, T44Q and Mn, derived from the neuronal MAP6D1 protein. The CD, TEM, ¹H-NMR, ITC and bioinformatics results show that T44Q consists of β -sheet and α -helical regions, whereas Mn only contains an α -helix. The secondary structure of T44Q remains stable up to 95 °C, while its tertiary structure changes. Contrary to Mn, T44Q peptides associate *in vitro*, forming amyloid-like aggregates. The immunofluorescence experiments show that, among the two peptides, only T44Q stabilizes microtubules against nocodazole in cells, whereas none of them against cold. The interaction between T44Q and calmodulin has a low affinity, being mostly Ca²⁺-independent. Mn interacts with tubulin or calmodulin through the same hydrophobic α -helical region. Our results might shed light on neuronal homeostasis and could serve for designing drugs against neuropathologies.

P-200

Anisotropic circular dichroism of macroscopically oriented light-harvesting complex II

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Light-harvesting complex II (LHCII) – a chlorophyll-protein complex in the chloroplast thylakoid membranes of plants and green algae – functions as a peripheral antenna of Photosystem II, accounting for up to 80% of the absorption cross-section. Coupling of transition dipoles of the tightly packed pigments in LHCII creates partly delocalized exciton states giving rise to circular dichroism (CD). Excitonic CD is highly sensitive to structural changes in the pigment-protein complexes. Anisotropic CD (ACD) of macroscopically oriented molecules and aggregates carries additional information about their structural organization, which is lost in the rotationally-averaged isotropic CD. Here we present ACD spectra of LHCII monomers and trimers embedded in lipid membranes, oriented as compressed gel slabs or surface-supported films. The ACD spectra showed drastically enhanced magnitude and level of detail compared to the corresponding isotropic CD spectra, revealing transitions that are too weak to resolve in absorption or CD spectra. Furthermore, by revealing the orientation of the transition dipole moments, ACD sets structural constraints to the possible assignments of spectral features and can be incorporated into a more complete and accurate structure-based exciton model of LHCII.

Posters

– 6. Protein structure to function –

P-201

Native mass spectrometry demonstrates the function of glycan attachment in viral infection pathwaysJ. Lockhauserbäumer¹, Y. Hao¹, H. Wegener², A. Mallagaray², S. Taube², T. Peters², G. Hansman³, C. Uetrecht^{1,4}¹Heinrich Pette Institute, Hamburg, Germany; ²Institute of Virology & Chemistry University of Lübeck, Germany; ³DKFZ & Department of Infectious Diseases & Virology University of Heidelberg, Germany; ⁴European XFEL, Germany

Glycans on human cells play an important role as attachment factors in viral infection pathways but the related entry processes are not well understood. In the presentation, details of the norovirus (NoV) glycan binding process such as stoichiometry, binding sites and glycan preference were followed with native mass spectrometry (MS). The technique is based on electrospray ionization (ESI), which allows soft ionization of target proteins and preserves non-covalent interactions. The main structure of interest for glycan contact is the protruding (P) domain of NoV. It is a part of the viral capsid that forms dimers. We analyzed the binding of various glycans to P dimers from several human NoV strains. At low glycan concentration crystal structures show the availability of two glycan binding sites. To get more information about the binding process, we increased the concentration to millimolar levels. Obtained MS data in conjunction with NMR experiments strongly suggest that cooperative multi step binding plays an important role in viral cell entry. MS results clearly reveal four glycan binding sites on the P dimer. Furthermore, recorded MS data show glycan preference and indicate glycan mediated structural changes in the P domain.

P-203

Biophysical and computational modelling tools to study RNA-protein complexesS. A. McKenna¹, M. Koch², J. M. Bujnicki³, J. Stetefeld¹, T. R. Patel⁴¹Department of Chemistry, University of Manitoba, Canada; ²Institute for Dental Research and Oral Musculoskeletal Biology, University of Cologne, Germany; ³International Institute of Molecular and Cell Biology, Poland; ⁴Alberta RNA Research and Training Institute, University of Lethbridge, Canada

RNA-protein and protein-protein interactions play crucial roles in many biological processes. To understand mechanisms underpinning such process, structural information is essential. The experimental determination of complexes that provides information on position and orientation of individual domains in many cases is not possible. Thus, high-resolution structures are not available for many complexes. Our laboratories employ an approach that combines low-resolution shape information about macromolecules and their complexes using biophysical methods, with high-resolution structures, and computational modeling to obtain atomic-level models. An application of such hybrid approach to study netrin-4/laminin gamma-1 complex, revealed that the N-terminal domains of proteins mediate interactions. Structural guided mutations provided insights on the biological relevance of this complex (Nat Commun 2016). A high-resolutions structural model between adenovirus VAI RNA and host enzyme, PKR was also investigated (J Struct Biol 2014) suggesting that the apical stem of VAI interacts with the RNA binding motifs of PKR.

P-202

The role of calcium in folding of the major pseudopilin PulG in the type 2 secretion systemA. Lopez-Castilla¹, J. L. Thomassin¹, B. Bardiau¹, E. Egelman², M. Nilges¹, O. Francetic¹, N. Izadi-Pruneyre¹¹Institut Pasteur, France; ²University of Virginia School of Medicine, USA

The type 2 secretion system (T2SS) is a bacterial secretion machinery used to translocate folded proteins from the periplasm to the extracellular medium. The pseudopilus is a periplasmic component of the T2SS, essential for secretion. It is assembled by the polymerization of the major pseudopili protein, PulG in *Klebsiella oxytoca*. The aim of this study is to obtain a high-resolution model of the pseudopilus structure to better understand the assembly and secretion process. To this purpose, the structure of its major protein component is a requirement. The PulG structure was previously determined by X-ray. However, it crystallized as a swapped dimer involving the C-terminal β -strand. Crystal structures of three other major pseudopilins showed a conserved Ca^{2+} -binding site in their C-termini followed by an α -helix, instead of the PulG swapped β -strand. These results suggest that PulG might bind Ca^{2+} as well, and its available X-ray structure would not reflect the protein conformation in the periplasm, where Ca^{2+} concentration can reach mM range. To determine the PulG structure in the presence of calcium, we used NMR. A cryo-EM map of the pseudopilus together with the NMR PulG structure were used to build a structural model of the T2SS pseudopilus.

P-204

Destructive effect of non-enzymatic glycation on catalase and remediation via curcuminF. Mofidi Najjar^{1,2}, F. Taghavi¹, R. Ghadari³, A. A. Moosavi-Movahedi¹¹University of Tehran, Iran; ²Shahid Beheshti University, Iran; ³University of Tabriz, Iran

Non-enzymatic glycation of protein is a post-translational modification which is produced by a covalent binding between reducing sugars and amino groups in lysine and arginine residues. In this paper, the effect of pathological conditions derived from hyperglycemia on catalase as a model protein was considered by measurements of the enzyme activity, reactive oxygen species (ROS) generation and changes in structural properties. It is observed that in the presence of glucose, catalase activity decreased gradually. ROS generation was also involved in the glycation process. So decrease in the BLC activity was considered as a result of ROS generation caused by glycation. However in the presence of curcumin, amount of ROS reduced, resulting in increase in glycated catalase activity. The effect of glucose and probably inhibitory effect of curcumin were also investigated on aggregation and structure changes of catalase.

Molecular dynamic simulation also showed that changes in structure of enzyme due to interaction with curcumin caused changes on accessible surface area (ASA) and pKa, which are effective parameters on glycation.

Decrease in ASA and increase in pKa in most likely glycation lysine residues were considered as predominant factor in decreasing in glycation amount.

Posters

– 6. Protein structure to function –

P-205

Protein S-sulfonation and S-thiosulfonation regulates non-enzymatic oxidative folding

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The incorporation of a disulfide bond into the secondary structure of proteins is a widely natural strategy to regulate protein nanomechanics. Importantly, disulfide rupture and reformation under force is a dynamic process regulated by the fluctuating changes in the chemical environment. In many instances, the attacking nucleophile remains bound to the protein upon disulfide scission giving rise to a post-translation modification (PTM). This ability of sulfur to undergo PTMs is due to the external empty d-orbital of sulfur ([Ne]3s²3p⁴3d⁰) available for bonding. While the general effect of mechanical forces on protein unfolding is now well understood, understanding how mechanical forces regulate the reduction and reformation of individual disulfide bonds remains largely elusive. Here we use a combination of protein engineering and single molecule force spectroscopy to investigate how different nucleophiles - sulfite and thiosulfate - regulate the reactivity of protein disulfide under force, with important knock-on effects for the nanomechanics of the protein. Our measurements reflect the dramatically different roles of the intimately related S-sulfonation and S-thiosulfonation on protein's elasticity, and we highlight the importance of force-induced exposure of cryptic disulfide bridges on the regulation of protein nanomechanics through PTMs.

P-207

Interaction between calmodulin and bisphenol A

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Calmodulin (CaM) is a high affinity intracellular Calcium receptor in Ca²⁺-dependent signaling in eukaryotic cells. CaM is responsible for converting the intracellular Ca²⁺ signal into a wide range of physiological events. The Ca²⁺ signal transduction is accomplished predominantly through Ca²⁺-induced conformational change of CaM. Bisphenol A (BPA) is suspected to affect reproductive toxicity and cell development. Pathological and behavioral abnormalities due to exposure of BPA have been reported. The effect of BPA on Ca²⁺ signaling through CaM may be also closely related to the developing schizophrenia. In this study we have investigated Ca²⁺ binding ability and structural change of CaM interacting with BPA using various scientific methods. We will present mechanism of interaction between CaM and BPA in detail.

P-206

Essential role of non-stochasticity in the cis-trans isomerization reaction in Pin1

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The importance of protein conformational heterogeneity in enzymatic reactions has become apparent, yet how it affects catalysis remains largely unclear. The studies of enzyme catalysis, on the other hand, have mostly focused on characterizing transition states and reaction kinetics, but understanding of reaction dynamics remains largely controversial. To this end, here we study Pin1 peptidyl-prolyl isomerase and discuss how conformational changes of Pin1 and ligand occur during the *cis-trans* isomerization reaction. Molecular dynamics simulations are used to calculate free energy profiles and the transition dynamics.

Our results show that the transitions do not follow the minimum free energy path, but rather occur rapidly such that proteins does not maintain thermal equilibrium with the ligand; thus protein-ligand interaction relaxes slowly after the isomerization.

The transitions are found to occur from a conformationally excited of protein.

These results indicate that non-stochastic dynamics in the equilibria play an essential role in catalyzing the *cis-trans* isomerizations in Pin1, which can be a general feature in broader enzymatic reactions.

P-208

Structural characteristics of S100B protein bound with divalent ions of Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺

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S100B is a member of a group of calcium-binding proteins, and is specifically expressed in astrocytes of glial cells in central nervous system. Structural characteristic of S100B bound with divalent ion of Mg²⁺, Zn²⁺, Cu²⁺ besides Ca²⁺ have also been investigated. Moreover, the relationship of structural properties of S100B protein bound these divalent ions with brain / neurological diseases have been pointed out. However, several questions remain in exploring the structural properties of S100B protein binding with other divalent ions. In this study, we focused on the structural characteristics of S100B protein binding with divalent ions of the second group elements of Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺. We will report the structural properties of S100B protein binding with these ions and the relationship between S100B and ionic radius of these divalent ions.

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– 6. Protein structure to function –

P-209

Thermodynamic study of off7-MBP interaction at different pHsM. Nemergut¹, E. Sedlák²¹Department of Biophysics, P. J. Šafárik University, Jesenná 5, 041 54 Košice, Slovakia; ²Center for Interdisciplinary Biosciences, P. J. Šafárik University, Jesenná 5, 041 54 Košice, Slovakia

DARPinS (designed ankyrin repeat proteins) represent a novel class of genetically modified proteins with antibodies-like characteristics. DARPins with specificity and high affinity to different ligands can be developed by display technologies and represent thus potentially new generation of protein therapeutics. In this project, we studied DARPins, namely off7, which was selected by ribosome display to specifically and with high affinity ($K_D = 4.4$ nM) to bind to maltose binding protein (MBP). The main aim of the project is to characterize the interaction of the off7 with MBP as well as to find out how is this interaction affected by different pH. The interaction of these two proteins was studied by isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). From the comparison of the results obtained by these techniques overall thermodynamic profile of this interaction can be acquired. The obtained results helped us to understand the mechanism of the protein-protein interaction under different conditions with an implication for biotechnological applications, such as affinity chromatography.

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P-211

Disulfide mapping the voltage-sensing mechanism of a voltage-dependent potassium channelT. Nozaki¹, S.-I. Ozawa¹, H. Harada¹, T. Kimura¹, M. Osawa^{1,2}, I. Shimada¹¹Graduate School of Pharmaceutical Sciences, the University of Tokyo, Japan; ²Faculty of Pharmacy, Keio University, Japan

Voltage-dependent K^+ (Kv) channels allow the selective permeation of K^+ in a membrane potential dependent manner, playing crucial roles in neurotransmission and muscle contraction. Kv channel is a tetramer, in which each subunit possesses a voltage-sensing domain (VSD) and a pore domain. Although several lines of evidence indicated that membrane depolarization is sensed as the conformational change of the VSD, the detailed voltage-sensing mechanism remained elusive, due to the difficulty of structural analyses at resting potential. In this study, we conducted a comprehensive disulfide locking analysis of the VSD using 36 double Cys mutants, in order to identify the proximal residue pairs of the VSD in the presence or absence of a membrane potential. The results strongly suggested that the VSD exists in a conformational equilibrium between up and down states, where helix S4 lies on the extracellular and intracellular sides of the membrane, respectively, in either a polarized or depolarized environment. Each state also exists in equilibrium among multiple conformations, where the axial rotation of S4 is allowed. The voltage dependent state transition is defined as the shift of the equilibrium between two states, each of which consists of multiple conformations.

P-210

Prediction of cancer-associated hotspot mutations that affect GPCR oligomerizationW. Nemoto¹, V. Limviphuvadh², S. Maurer-Stroh², Y. Yamanishi³, M. Yamanoi¹, H. Toh⁴¹Department of Science and Technology, Tokyo Denki Univ., Hatoyama-machi, Japan; ²Bioinformatics Institute, Agency for Science, Technology and Research, Singapore; ³Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; ⁴School of Science and Technology, Kwansei Gakuin University, Sanda-shi, Japan

We developed a high-performance method to predict interacting pairs for G-Protein Coupled Receptors (GPCRs) oligomerization, GPCR-GPCR Interaction Pair predictor (GGIP), by integrating the structure and sequence information [Nemoto et al. *Proteins*. 2016;84:1224-33]. A recent study reported that somatic mutations in GPCR-encoding genes are frequently found in various types of cancer. Among the somatic mutations, hotspot mutations are defined as recurrent amino acid changes occurring in coding sequences. It has been revealed that many GPCRs have hotspot mutations in the same types of cancer tissues. However, most of the hotspot mutations have not been characterized yet, and their effects on the cancer pathways remain unknown. Some of the hotspot mutations may be related to cancers through modifying GPCR oligomerization, since they are considered to be present on the surface of transmembrane helices. Hence, we examined the predicted interacting pairs including the GPCRs with hotspot mutations. We will discuss the characteristics of these mutations and introduce several examples. Our prediction server is available at <http://protein.b.dendai.ac.jp/GGIP/>.

P-212

Large production of mammalian membrane proteins toward determination of high-resolution structure

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Despite the large increase in the rate of deposition of membrane protein structures in the PDB, the number of unique structures of mammalian ones is still less than 100 as of September, 2016 (less than 0.1% of the number of the total depositions). One of the reasons is that there is no easy as well as versatile system for the production of membrane proteins. Here we present our recombinant adenovirus expression system, which is robust and easy to handle. We have already succeeded in producing more than 10 unique mammalian membrane proteins. In the case of sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA), 4 mg of purified protein was obtained from 1 L culture. SDS-PAGE with CBB staining of the purified protein gave a nearly single band at the expected position. The specific activity of the recombinant protein was comparable to that of native protein purified from SR. This amount of purified protein is sufficient for crystallization trials. Indeed, crystals in $E1Mg^{2+}$ state diffracted to 3.2 Å resolution.

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– 6. Protein structure to function –

P-213

Lipase-specific foldases are steric chaperones that act as dynamic folding platformsK. Pauwels^{1,2}, H. H. Nguyen^{1,2}, N. Bata^{1,2}, P. Lebrun^{1,2}, P. Tompa^{1,2}¹VIB, Centre for Structural Biology, Pleinlaan 2, 1050 Brussels, Belgium; ²VUB, Structural Biology Brussels, Pleinlaan 2, 1050 Brussels, Belgium

Many proteins contain disordered regions that only fold when they bind to a partner molecule. The bacterial lipase-specific foldases (Lifs) are crucial for the folding and activation of their cognate lipase (LipA). The decisive step in the LipA biogenesis is catalyzed by Lif, since in the absence of this steric chaperone, LipA cannot fold autonomously into its biologically active and secretion-competent conformation.

Despite having solved the crystal structure of the *Burkholderia glumae* LipA-Lif complex, the exact action mechanism of Lifs remains enigmatic. Therefore we also studied the *Pseudomonas aeruginosa* Lif (LipH) and its cognate lipases, LipA and LipC. By combining various biophysical techniques like optical spectroscopies, bilayer interferometry, limited proteolysis, NMR and SAXS we show that Lifs are partially intrinsically disordered proteins that experience a conformational rearrangement upon binding their substrate. We propose that free Lifs contain at least one minidomain that functions as stable scaffold for docking of LipA and we have identified a flexible segment that undergoes a contact-assisted structure formation when Lif binds its lipase.

P-215

Analysis of conformational properties of AsLOV2 domainM. Petrenčáková¹, D. Jancura^{1,2}, E. Sedlák²¹Department of Biophysics, P. J. Šafárik University, Košice, Slovakia; ²Center for Interdisciplinary Biosciences, P. J. Šafárik University, Košice, Slovakia

Phototropins are blue light photoreceptors which contain two Light-Oxygen-Voltage (LOV) domains, LOV1 and LOV2. LOV domains non-covalently bind flavin chromophore. These relatively small domains function as light sensors and sensitively respond to irradiation with blue light which induces a conformational change, completely reversible in the dark. LOV domain thanks to its light induced conformational change has potential widespread use in optogenetics, i.e. as molecular switch in cell signaling. It can also be used as a fluorescent protein when their advantage over GFP is smaller size, utility under anaerobic conditions and their ability to generate reactive oxygen species. Here, we present our findings regarding thermodynamic properties of AsLOV2 from *Avena sativa* obtained by CD, fluorescence spectroscopy, UV-Vis spectroscopy and differential scanning calorimetry. We show that conformation of AsLOV2 sensitively reacts on pH changes in the neutral pH region. Thermal stability studies indicate that AsLOV2 exist in metastable form, i.e. AsLOV2 can switch to more stable form by high temperature. We believe that the obtained results might be of great interest in the rational design of light sensitive conjugates containing AsLOV2 domain.

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P-214

Applications of non-covalently bound membrane proteins in nanodiscs using LILBID-MS

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Membrane protein complexes are particularly challenging to investigate with Mass Spectrometry (MS) as they require e.g. detergents for solubilization. However some complexes are not stable in detergents. For those, we focus on nanodiscs (ND) as carrier of those complexes, consisting of lipids which mimic the lipid bilayer of the membrane. We use our homebuilt LILBID-MS (Laser-Induced-Liquid-Bead-Ion-Desorption) combined with activity assays to investigate membrane proteins embedded in ND.

In LILBID, a piezo-driven droplet generator generates droplets of 30 micrometer diameter, which are transferred to high vacuum, irradiated by an IR laser operating at 2.94 micrometer, leading to an explosive expansion of the droplet which releases the solvated analyte ions [1].

Recently we showed that LILBID-MS is able to reveal the oligomeric state of membrane proteins directly from ND [2]. The activity/functionality of many membrane proteins depends on the lipid induced oligomeric state as we demonstrate for the translocase MraY [2]. For the zinc-transporter SV31 we could prove that mutation of the active domain has no influence on the oligomeric state, which we determined to be dimeric [3].

[1] Morgner et al., *Austr. J. Chem.*, 59:109–114, 2006.[2] Peetz et al., *eLife* 2017;6:e20954.[3] Waberer et al., *J Neurochem*, p. 1-14, 2016.

P-216

Probing the unique molecular mechanisms used by *Clostridium thermocellum* for Cellulosome functionB. A. Pinheiro¹, J. L. Brás^{2,3}, P. Bule², V. M. Pires², M. J. Romão¹, V. D. Alves², A. S. Palma¹, C. M. Fontes^{2,3}, A. L. Carvalho²¹UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; ²CIISA/FMV-UL, Faculdade de Medicina Veterinária, Universidade de Lisboa, Portugal; ³NZYTech Genes & Enzymes, Campus do Lumiar, Estrada do Paço do Lumiar, Edifício E, r/c, 1649-038 Lisboa, Portugal

Some cellulolytic bacteria are remarkably efficient in the degradation of the intricate network of polysaccharides that constitute the plant cell wall. The driving force is the assembly of multi-enzyme complexes of catalytic and non-catalytic modules that work together as a megaDa machinery: the Cellulosome. X-ray crystallography is the methodology that is providing structural information on the interactions of these modules, either protein-protein^{1,2,3} or protein-carbohydrate⁴ and about the catalytic sites of the enzymes⁵. Crucial information that guides the production of complexes of interest can be given by microarray analysis⁶. Joining all the information, we are elucidating the unique molecular mechanisms for cellulosome cellular attachment, assembly and activity. 1-*PNAS* **100**, 13809–13814 (2003) 2-*PNAS* **104**, 3089–3094 (2007) 3-*Sci. Reports* **6**, 38292 (2016) 4-*JBC* **281**, 8815–8828 (2006) 5-*PNAS* **108**, 5237–5242 (2011) 6-*Mol. Cell. Proteomics* **14**, 974–88 (2015)

Posters

– 6. Protein structure to function –

P-217

Structural basis of anti-PD-L1 monoclonal antibody avelumab for tumor therapy

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The crystal structures of PD-L1 complexed with its receptor PD-1 have been extensively studied, including human PD-L1 (hPD-L1) alone, mouse PD-1 (mPD-1) complexed with hPD-L1 and human PD-1 (hPD-1) complexed with hPD-L1. However the complex structure of hPD-L1/mAb complex structure has not been investigated. We crystallized crystal structure of avelumab-scFv/ hPD-L1 complex. It is speculated that three competitive binding patterns of the antibody would lead to the blockade of paired molecules to bind each other, i.e., neighboring non-overlapping binding, partially overlapping binding and completely overlapping binding. The blocking mechanism of the avelumab belongs to the partially overlapping pattern. Our findings would benefit the design and optimization of therapeutic antibodies targeting hPD-L1.

P-219

Structural and functional studies of drug targets for *Mycobacterium tuberculosis*Z. Rao^{1,2}¹Institute of Immunochemistry, Shanghai Tech University, China; ²Laboratory of Structural Biology, Nankai University, China

Around 1/3 of world population, ~2 billion people, are thought to be infected with *Mycobacterium tuberculosis* (MTB), which results in 1-2 million fatalities per year. One of the great concerns is that the increasing prevalence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) renders many of the current treatments ineffective.

In order to overcome these problems, it is essential to identify new drug targets involved in the essential physiological processes and have a complete understanding about the drug resistance development in MTB. We initiated a project to carry out extensive structural and functional studies on the drug targets involved in DNA replication, cell wall synthesis, immune escape, metabolism pathways and drug resistance in this pathogen. Using crystallography, cryo-EM and biochemical methods, we have obtained structural and functional data for dozens of new drug targets participating in the above important physiological processes, which will have great potential in developing new therapies against mycobacteria infection.

P-218

Structural Virology Studies in Zihe Rao's groupZ. Rao^{1,2}¹Institute of Biophysics, Chinese Academy of Science, China;²Laboratory of Structural Biology, Tsinghua University, China

Viral diseases are serious public health threats and have caused considerable concerns over the public health safety. Despite the outnumbering cellular forms of life, our knowledge of viral life cycle remains limited. The Rao group applies a combination of structural analysis, cellular assays, reverse genetics and animal studies to understand the molecular mechanisms involved in the various steps of virus cycle, including entry, uncoating and replication. During the past few years, we have determined the structures of proteins involved in viral replication (Avian influenza polymerase complex, SARS-CoV nsp5, nsp7/8, nsp10/14 complexes) and viral-receptor interaction (EBOV GP, SCARB2, TIM-1, NPC1); in addition, we also solved a number of viruses (EV71, CVA16, HAV, AiV, PV, JEV, ZIKV, HSV capsids...) and virus-antibody complexes (EV71-D6, EV71-A9, HAV-R10, JEV-2F2, JEV-2H4...) by crystallography or electron microscopy. These structures allow us to design better antiviral compounds and vaccines to translate our research findings into public health benefits.

P-220

Do encapsulated polyphenols combat oxidative stress? A case study with Ribonuclease A

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Aging in living cells is a direct consequence of oxidative stress in proteins. One of the widely used markers in oxidative stress is the formation of dityrosine (DT) cross-linkages in proteins. Ribonuclease A (RNase A) cleaves RNA in order to maintain an optimal balance in protein synthesis. RNase A shows a characteristic DT fluorescence peak upon oxidation using potassium persulfate and the cobalt (II) ion. Apart from dimer formation, the protein undergoes major changes in its secondary structure. Polyphenols (GA, ECG and EGCG) shows strong antioxidant activity and hence are used to prevent oxidation in RNase A. Although ECG prevents DT formation to a large extent, it promotes protein oligomerization along with EGCG due to protein-polyphenol cross-linking. On the other hand a β -cyclodextrin (β -CD) encapsulated polyphenols prevents protein oligomerization as well as DT formation. This may be attributed to the fact that the quinone forming rings of ECG and EGCG are encapsulated in the cavity of β -CD and hence no longer available for protein cross-linking.

Posters

– 6. Protein structure to function –

P-221

Understanding the interplay between amino acid sequence and water in collagen mimetic peptides

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Collagen is the most abundant protein in mammals and provides structural strength to tissues. Recent studies have shown that collagen fibers undergo osmotic pressure-induced structural changes, which result in molecular contraction. We hypothesize that local, sequence-dependent interactions with water are responsible for this effect. Using collagen mimetic peptides, we aim to understand the sequence-structure-mechanics relationship of these osmotic pressure induced conformational changes. The triple helix-forming peptides (ProProGly)₁₀, (ProHypGly)₁₀, (HypHypGly)₁₀ serve as our model system. They exhibit different surface properties resulting from different proline (Pro) vs. hydroxyproline (Hyp) contents. Using infrared spectroscopy, we show that the strength of triple helix-stabilizing hydrogen bonds increases when increasing the osmotic pressure. X-ray scattering shows that the osmotic pressure affects the helix pitch as well as the packing distance between helices. Even though all peptides are affected in a similar way, the magnitude of the observed change is different and correlates with the surface properties. These results highlight how helix surface properties influence the interaction with surrounding water, thereby altering the response of collagen to osmotic stress.

P-223

Biophysical study and inhibitory effect of toluidine blue O on lysozyme amyloid fibrillogenesis

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Binding interaction of photoactive organic small molecules with proteins has evoked great interest in medicinal chemistry. Many disorders like Alzheimer's, Parkinson's, type II diabetes etc are caused by protein aggregation. A detailed study of dye-protein interactions may help to understand the structural features, bio-affinity and pharmacokinetic behavior of dye on protein domain. The intrinsic fluorescence of lysozyme by TBO provided information about the exposed Trp-62 and 63 residues. The interaction caused significant loss in lysozyme helicity and alterations in the microenvironment surrounding the tryptophan and tyrosine residues also occurred. The binding was exothermic and driven by positive entropy changes with minimum favourable enthalpy changes. The amyloid fibril formation was also examined. TBO was found to delay lysozyme fibrillation and had a significant inhibitory effect on fibrillogenesis. Understanding on the binding aspects of small molecules to lysozyme provides information to develop better small molecule inhibitors in amyloid diseases therapy.

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Ion selectivity at low affinity: exploring potassium binding by Kbp by NMR, SAXS and simulation

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Potassium is key to cellular homeostasis. *E. coli* possesses several specific K⁺ influx and efflux systems that maintain the intracellular K⁺ concentration over a broad range of external pH, osmolarity and K⁺ concentrations. Although regulatory proteins and sensor domains have been identified for a number of these K⁺ ion transport systems, the exact mechanism through which K⁺ concentration is sensed in the cell and therefore how these systems are regulated remains unknown.

The cytoplasmic protein Kbp is one of a small number whose expression is strongly upregulated in response to osmotic stress. We discovered that Kbp is an unprecedented highly selective, soluble K⁺ binding protein and determined its K⁺-bound structure (1). NMR, CD and ITC studies of Kbp show that K⁺ binding induces a conformational change in Kbp that orders the protein structure that cannot be brought about by even high concentrations of Na⁺. We have used a range of techniques to explore the conformational repertoire of Kbp and identify the ion binding site.

(1) Ashraf et al (2016) Structure 24:741-749.

P-224

Investigating protein dynamics from multiple crystallographic structures

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Crystallographic structures are, by their nature, averaged representatives of a proteins lowest vibrational energy state. This has allowed for unrivalled resolution of a proteins three-dimensional structure, enabling vast leaps across a wide range of biological research. However, the nature of crystal structures do not allow for an advanced view on the dynamic behavior of proteins in solution. Using the model protein, Catabolite Activator Protein (CAP), we have shown that different crystallographic forms of a protein captured may be representative 'snap-shots' of a proteins native motions in solution. Combining normal mode analysis with a novel visualization method provide an argument for the utilization of molecular dynamics to understand a proteins native structure in a more native and dynamic form.

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– 6. Protein structure to function –

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***In vitro* reconstitution of inhibitory GABAergic postsynapses**

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The organisation of GABAergic inhibitory postsynapses is strongly regulated and depends on multiple proteins. Hence, the assembly process is prone to dysfunction resulting in diseases like mental retardation, autism and epilepsy. To understand the mechanism underlying receptor organisation, the interactions of the individual proteins need to be investigated. The assembly process critically depends on gephyrin, which is recruited to the postsynaptic membrane by the adaptor protein collybistin (CB) that conformationally switches from a self-associated, inactive state to an open one. In cells, this conformational switch is supposed to be induced by interaction with neuroligin-2.

To gain insight in postsynaptic density formation, we started to investigate the activation of collybistin focussing on its interaction with receptor lipids in the postsynaptic membrane. This is based on previously found affinities of CB to phosphoinositides (PI).

On this account we work on different solid supported membrane systems doped with varying PIs to detect conformational changes in CB isoforms by either RfFS or AFM. The correlation of an optical, non-invasive method and a mechanical method will facilitate a determination of factors influencing the neurotransmitter receptor organisation.

P-227

AFM-based Single-Molecule Force Spectroscopy on monovalent streptavidinS. M. Sedlak^{1,2}, M. S. Bauer^{1,2}, F. Baumann^{1,2}, L. C. Schendel^{1,2}, C. Kluger^{1,2}, D. A. Pippig^{1,2}, H. E. Gaub^{1,2}¹Chair of Applied Physics, LMU München, Germany;²Center for NanoScience, LMU München, Germany

With femtomolar affinity, the binding of biotin to the homotetramer streptavidin is one of the strongest non-covalent biological interactions known. In bionanotechnology, it is commonly used for detection, trapping or purification of biomolecules. Being one of the first receptor-ligand systems studied by single-molecule force spectroscopy (SMFS), the interaction between biotin and tetravalent streptavidin has been intensively examined. The tetravalency of streptavidin yet results in undefined binding and tethering geometries, which impede the measurement of monodisperse data.

We use an engineered monovalent version of streptavidin and specific tethering strategies for site-selective immobilization to overcome these problems. To confirm single rupture events of biotin:streptavidin, we make use of biotinylated proteins with known unfolding patterns. We perform high-quality AFM-based SMFS measurements with a well-defined stoichiometry and probe this receptor-ligand system in a controlled manner under various pulling geometries. Our results enable us to understand the cause of the binding strength of this system in detail. In addition, comparing the mechanical properties of streptavidin with related systems provides deeper insight into the relation of binding forces and affinities.

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Reconstitution of silicanin-1 into artificial lipid membranes and its function for silica biogenesis

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Silica cell wall formation of the micro algae group called diatoms takes place in the silica deposition vesicle (SDV) and the silica structures are finally discharged via exocytosis. The SDV has not been successfully isolated yet. Therefore, little is known about its lipid composition and properties.

Working with a model membrane mimicking the silicalemma of the SDV the interaction of this membrane with biosilica associated molecules and its influence on silica precipitation is investigated *in vitro*. In context of cell wall dissolution many soluble biosilica associated molecules like silaffins and long-chain polyamines are obtained amongst an insoluble organic matrix. This matrix can be dissolved and yield cingulins and silicanins. The latter are found to be the first group of transmembrane proteins associated to biosilica. Silicanin-1 (Sin1) is a representative of this group for the diatom species *Thalassiosira pseudonana* and its role for silica biogenesis is examined. The reconstitution of Sin1 into artificial lipid membranes is followed by the investigation of its self-aggregation behavior and silica precipitation properties by means of atomic force and fluorescence microscopy as well as reflectometric interference spectroscopy.

P-228

Structural principles for engineering nitrilasesB. T. Sewell¹, A. E. Mulelu¹, J. Reitz², A. Frangakis²¹University of Cape Town, South Africa; ²Goethe University, Frankfurt, Germany

Cyanide degrading enzymes are typical nitrilases - their unique property is the conversion of hydrogen cyanide to formic acid and ammonia. A growing body of evidence indicates that nitrilase monomers must associate in order to gain activity. Typically the monomers associate in two previously identified interface regions (called the A and C surfaces) to form spiral assemblies. We report here on the determination of the helical structure of the cyanide dihydratase (CynD) from *Bacillus pumilus* that is formed at pH5.4. The structure was determined by cryo-electron microscopy and has a resolution of 3.2 Å. The electric potential map enables clear visualization of residues 3 – 319 of a total of 330 residues per monomer. The C-terminal “tail”, that is essential for activity, lies on the inside of the helical structure and interacts with three monomers other than the one from which it arises. This helps to stabilize the inter-subunit interactions and suggests many strategies for increasing the stability of the enzyme for industrial use. Quantum mechanical modeling based on the structure of the active site provides a means for distinguishing between the competing mechanistic proposals and may ultimately lead to rational design of nitrilase specificity.

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– 6. Protein structure to function –

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Characterizing structure-dynamics of native and pH induced partly folded conformation of T7 endolysinM. Sharma¹, D. Kumar², K. M. Poluri¹¹Indian Institute of Technology Roorkee (IITR), India;²Centre of Biomedical Research (CBMR), India

T7 bacteriophage endolysin is a single-domain zinc containing amidase that cause rapid lysis of Gram-negative bacteria. It is reported that they show differential activity in the pH range 7–3. However, the molecular basis for such pH-dependent differential activity is not known. In this study biophysical investigations have been done on T7L to delineate the pH induced structural fluctuations. Our studies established a reversible structural transition of T7L and formation of partially collapsed conformation around pH 5–3. To obtain mechanistic insights of such conformational transitions we have assigned NH cross-peaks of native and partly collapsed conformations using NMR spectroscopy. pH dependent HSQC experiments confirmed that the native state at pH 7 is heterogeneous and decrease in pH below 6 results in partially folded conformation. Partially collapsed structure is thermally stable with exposed hydrophobic pockets and exhibits complete structure-function reversibility. NMR relaxation parameters of native and partly folded conformations were analyzed to correlate polypeptide chain motions. Our comprehensive study concluded that a coupled network of His residues is responsible for reversible pH dependent conformational dynamics, structural transition and differential activity.

P-231

Complex structure of cytochrome *c*–cytochrome *c* oxidase reveals a novel interprotein interaction modeK. Shinzawa-Ittoh¹, S. Shimada¹, J. Baba¹, S. Aoe¹, A. Shimada¹, E. Yamashita², J. Kang¹, M. Tatenio¹, S. Yoshikawa¹, T. Tsukihara^{1,2}¹Graduate School of Life Science, University of Hyogo, Japan; ²Institute for Protein Research, Osaka University, Japan

Mitochondrial cytochrome *c* oxidase (CcO) transfers electrons from cytochrome *c* (Cyt.*c*) to O₂ to generate H₂O, a process coupled to proton pumping. To elucidate the mechanism of electron transfer, we determined the structure of the mammalian Cyt.*c*–CcO complex at 2.0 Å resolution and identified an electron transfer pathway from Cyt.*c* to CcO. The specific interaction between Cyt.*c* and CcO is stabilized by only six electrostatic interactions between side chains within a small contact surface. Between the two proteins are three water layers with a long inter-molecular span, one of which lies between the other two layers without significant direct interaction with either protein. Cyt.*c* undergoes large structural fluctuations, using the interacting regions with CcO as a fulcrum. These features of the protein–protein interaction at the docking interface represent the first known example of a new class of inter-protein interaction, which we term “soft and specific”. This interaction is likely to contribute to the rapid association/dissociation of the Cyt.*c*–CcO complex, which facilitates the sequential supply of four electrons for the O₂ reduction reaction.

P-230

Structural insights into small GTPases regulation by SmgGDSH. Shimizu¹, S. F. Toma¹, N. Shimizu³, S. Saijo³, K. Kontani^{2,1}, T. Katada¹, T. Shimizu¹¹Grad. Sch. of Phar. Sci., The Univ. of Tokyo, Japan;²Phar. Sci., Meiji Phar.Univ., Japan; ³SBRC., PF, IMSS, KEK, Japan

SmgGDS is a guanine-nucleotide exchange factors (GEF) for RhoA and is supposed to have novel GEF mechanism, because SmgGDS has no canonical GEF domain and it is composed of armadillo repeat motifs (ARMs) only. SmgGDS has two splicing variant isoforms; SmgGDS-558 and -607. These isoforms considered to have different physiological role. Interestingly, SmgGDS can interact with many types of small GTPases possessing poly basic region (PBR). It is proposed that SmgGDS works also as molecular chaperon to regulate small-GTPase trafficking and localization. Here we determined the crystal structure of SmgGDS-558 and the SAXS solution structures of SmgGDS-558 and its complex with RhoA. Comparison of these structures clarified that both SmgGDS variants held RhoA at their concave surfaces. Electrostatic potential map of SmgGDS-558 revealed characteristic acidic and basic areas on its molecular surface. Using SmgGDS mutants, we performed binding assay and GEF activity assay and revealed that SmgGDS had two major small GTPase binding sites and each splice variant had their own binding manner for small GTPases and change their GEF activity depending on lipidation state. This is the first detailed report to demonstrate a relationship between molecular function and structure of SmgGDS.

P-232

Self-assembly of Influenza A virus protein scaffold: interplay of structure and functionE. V. Shtykova⁴, A. S. Lyushnyak^{1,2}, L. A. Shilova^{1,2}, N. V. Fedorova³, D. G. Knyazev⁵, T. R. Galimzyanov¹, O. V. Batishchev¹¹Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Federation; ²Moscow Institute of Physics and Technology, Russian Federation; ³Belozersky Institute of Physico-Chemical Biology, Russian Federation; ⁴Shubnikov Institute of Crystallography, FSRC “Crystallography and Photonics”, Russian Federation; ⁵Johannes Kepler University Linz, Institute of Biophysics, Austria

Influenza matrix protein M1 is known to possess multifunctionality. Besides viral scaffold formation, it acts as a crucial factor for different processes, such as the release of viral RNA during infection and the budding of newly assembled virions. These processes take place at different pH conditions; therefore, it is of great interest to investigate the structural changes of M1 protein itself and viral protein scaffold under pH changing. We combined studies of M1 protein using small angle X-ray scattering with atomic force microscopy and confocal microscopy to investigate processes of the protein self-assembly in the physiologically relevant range of pH, from 4 to 7. We revealed the tendency of M1 protein to form helical structures, even in acidic medium, and found the conditions required for M1 to perform the last stage of viral infection: budding of progeny virions.

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– 6. Protein structure to function –

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Fine-tuning of a tryptophan radical revealed by EPR spectroscopy: from structure to functionG. Sicoli^{1,4}, J.-M. Mouesca¹, L. Zeppieri², P. Amara², L. Martin², A.-L. Barra³, J. C. Fontecilla-Camps², Y. Nicolet², S. Gambarelli¹¹Université Grenoble Alpes (UGA), CEA/CNRS, INAC-SYMMES, Grenoble, France; ²Metalloproteins Unit, Institut de Biologie Structurale (IBS), CEA/CNRS/UGA, Grenoble, France; ³Lab. Nat. des Champs Magnétiques Intenses, UPR CNRS 3228, France; ⁴LASIR, UMR CNRS 8516, Univ. de Lille 1, Sciences et Technologies, Villeneuve d'Ascq, France

The radical *S*-adenosyl-*L*-methionine tryptophan lyase NosL converts *L*-tryptophan into 3-methylindolic acid, a precursor in the synthesis of the thiopeptide antibiotic nosiheptide. Identification of shunt products and homology with the FeFe-hydrogenase maturase tyrosine lyase HydG suggest a fragmentation-recombination mechanism that implied the C α -C β bond cleavage of *L*-tryptophan. Using CW and advanced pulsed EPR spectroscopic techniques and different *L*-tryptophan isotopologs, we trapped and characterized unanticipated radical intermediates that rule out this proposal (G. Sicoli et al. *Science* **2016**, *351*, 1320-1323; *Science* **2016**, *351*, 1266-1267). These radical species are evidence of an unprecedented carboxyl fragment migration. We deciphered subtle substrate motions in NosL active site, responsible for a fine-tuned radical chemistry. Exhaustive EPR studies combined with DFT calculations have shed light on different structural aspects strictly related to the bio-chemical synthetic aspects. Our data allow for the full description of the catalytic mechanism for this enzyme. Interesting highlights on different mutations of this enzyme are also provided.

P-235

Near-atomic structure of alternative complex III from *Rhodothermus marinus* by single-particle cryoEMJ. S. Sousa¹, F. Calisto², D. J. Mills¹, P. N. Refojo², M. Teixeira², M. M. Pereira², J. Vonck¹¹Department of Structural Biology, MPI of Biophysics, Germany; ²ITQB, Universidade Nova de Lisboa, Portugal

The respiratory electron transport chain is responsible for the generation and maintenance of the proton gradient used by ATP synthase in the production of ATP. Its canonical electron transfer complexes have been extensively studied in the last decades and it has also been known that in several organisms different enzymes ensure the electron flow in specific sections of the chain. Nonetheless, cytochrome *bc*₁ complexes were, until recently, the only known family of quinol:cytochrome *c* oxidoreductases.

Alternative Complex III (ACIII) is a member of a family of membrane-bound enzymes with quinol:HiPIP/cytochrome *c* oxidoreductase activity, widespread in the Bacteria domain. Its coding genes coexist in some cases with those coding for the classical complex III.

The 270 kDa ACIII is unrelated to the cytochrome *bc*₁/*b*₆*f* complexes and consists of four integral transmembrane subunits, two cytochrome *c* subunits and a peripheral subunit with four predicted iron-sulfur clusters. In order to unravel the catalytic mechanism of this novel family of electron transfer complexes, a structural insight of ACIII is fundamental. We used single-particle cryo-EM to determine the structure of ACIII from *Rhodothermus marinus* at near-atomic resolution.

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An arginine residue in flagellin as the hot spot of TLR5 binding and activationW. S. Song¹, Y. J. Jeon¹, B. Namgung¹, M. Hong², S. I. Yoon¹¹Kangwon National University, South Korea; ²Yonsei University, South Korea

Flagellin is the principal component of the flagellar filament that is essential for bacterial motility and pathogenicity. In mammals, flagellin is a major factor that evokes the innate immune response against pathogenic bacteria by interacting with Toll-like receptor 5 (TLR5). Although TLR5 is reported to respond to flagellins from Gram-negative β - γ -proteobacteria and Gram-positive Firmicutes bacteria, it is unknown how diverse flagellins with variations in sequence and domain organization stimulate TLR5. Based on structural, biochemical, and cellular studies of flagellin-mediated TLR5 activation, we present the common mechanism of the flagellin-TLR5 interaction. The crystal structure of a complex between *Bacillus subtilis* flagellin (*bs*flagellin) and TLR5 at 2.1 Å resolution, combined with exhaustive alanine scanning, reveals that Arg89 and its adjacent residues in the D1 domain of *bs*flagellin protrude into a cavity generated by the LRR9 loop of TLR5 and form a hot spot for TLR5 interaction and activation. The sequence and structure of the hot spot residues are highly conserved across TLR5-activating flagellins but not in TLR5 non-activators. These results would shed light on the future design of flagellin-based therapeutics.

P-236

Cytoprotective bile acids inhibit Bax activityT. Sousa¹, Š. Pokorná², N. Bernardes³, R. E. Castro⁴, M. Hof², S. Banerjee⁵, R. J. Youle⁵, A. I. A. Coutinho¹, C. M. P. Rodrigues⁴, M. Prieto¹, F. Fernandes¹¹CQFM and IN, IST, UL, Portugal; ²J. Heyrovský Institute, Acad. of Sci. of the Czech Republic, Czech Republic; ³IBB, IST, UL, Portugal; ⁴Med.U.Lisboa, FFUL, UL, Portugal; ⁵Surgical Neurology Branch, NINDS, NIH Bethesda, MD 20892, USA

The bile acids UDCA and TUDCA, have been shown to inhibit apoptosis in cells. The mechanisms associated with these effects are not entirely clear. However, the effect of hydrophilic bile acids seems to be related with the blockage of processes that converge on mitochondrial damage. Bax is a pro-apoptotic protein that is involved in pore formation on mitochondrial membranes. Cytoprotective bile acids have been shown to modulate Bax concentration in mitochondria, suggesting a possible direct interaction with the protein. Here, we aimed to characterize the impact of the interaction of Bax with physiologically active concentrations of UDCA and TUDCA on Bax activity, oligomerization, and mitochondrial membrane affinity, making use of fluorescence methodologies. We show that cytoprotective bile interact with Bax stabilizing the monomeric specie. Also, we observed that cytoprotective bile acids inhibit the interaction of the protein with activator peptide BH3, and with membranes mimicking the MOM. Finally, cytoprotective bile acids inhibit the pore forming activity of Bax on both LUVs and GUVs. Supported by FCT/Portugal' project FAPESP/20107/2014 and grants SFRH/BD/92398/2013 (TS) and IF/00386/2015 (FF).

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– 6. Protein structure to function –

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The functional characterization of the heterologous acid phosphatase from *Trichoderma harzianum*

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Trichoderma harzianum is a saprophytic fungus known for its potential as a biological control agent and its crucial role in the release of carbon, nitrogen and phosphorus to the environment. Phosphorus is released through the action of phosphatases, converting organic phosphate into a soluble inorganic form by hydrolysis. This is important for the recycling of organic phosphate and bioremediation process. In the present study, an acid phosphatase (ACPase) from *T. harzianum* was expressed in *Pichia pastoris* and functionally characterized. ACPase purified presented 22% of glycosylation and a molecular mass of 85 kDa. The optimum pH and temperature were 4.0 and 70°C. ACPase has a Km of 0.033 μ M and Vmax 11.17 μ M min⁻¹ to pNP-P substrate. Interestingly, this enzyme was shown to bind specifically to phytic acid. The activity was highly inhibited by KH₂PO₄ and sodium tungstate. ACPase was susceptible to broad pH variation but enzyme remained stable between 25 to 95°C at pH 4.0, in which it was shown to be organized into monodisperse tetramers. ACPase presents a conserved catalytic site and secondary structure. In sum, the heterologous enzyme is similar to the native enzyme and can be applied as biotechnological tool for removing phosphate from soils in the processes of bioremediation.

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Protein structure determination in living eukaryotic cells by in-cell NMR spectroscopy

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In-cell NMR is currently the only approach that can provide structural information of proteins inside cells at atomic resolution. Extending in-cell NMR to study proteins inside higher eukaryotic cells, and thus making this method more useful in medical and pharmaceutical researches, was an important issue to be investigated. Various methods have been established for in-cell NMR studies in human cultured cells. However, the low intracellular concentration of target proteins in HeLa cells makes it difficult to perform detailed NMR analyses.

We will report our recent methodological developments in stable isotope labelling of target proteins, extension of lifetime of cells by using the BIOREACTOR system. We would also report our recent in-cell NMR studies on obtaining pseudocontact shifts (PCSs) and paramagnetic relaxation enhancements (PREs) from protein-tagged paramagnetic lanthanoid ions, which can be analysed using sensitive 2D heteronuclear correlation NMR spectra. In-cell PCS/PRE experiments offer huge potential advantage in obtaining structural restraints over conventional NOE-based approaches, thus providing the useful information enabling the determination of protein structures, relative orientations of domains and protein complexes within human cultured cells.

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Photoactivation reduces side-chain dynamics of a LOV photoreceptor

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We used neutron scattering experiments to probe the conformational dynamics of the light, oxygen, voltage (LOV) photoreceptor PpSB1-LOV from *Pseudomonas putida* in both the dark- and light-state. Protein dynamics were measured using incoherent neutron time-of-flight and backscattering spectroscopy on the picosecond to nanosecond time scales. Mean square displacements of localized internal motions and effective force constants $\langle k' \rangle$ describing the resilience ('stiffness') of the proteins were determined on the respective time scales. Photoactivation significantly modifies the flexibility and the resilience of PpSB1-LOV. For a residue resolved interpretation of the experimental neutron scattering data we analyzed MD simulations of the PpSB1-LOV X-ray structure. Based on this data, it is tempting to speculate that light-induced changes in the protein result in altered side-chain mobility mostly for residues on the protruding J-alpha helix and on the LOV-LOV dimer interface. Our results provide strong experimental evidence that side-chain dynamics play a crucial role in photoactivation and signaling of PpSB1-LOV via modulation of conformational entropy. Stadler et al. Biophysical Journal, 2016, 1064-1074

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Structural insights and the low mechanical stability of the Au-S bond in gold-specific protein GolB

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The coordination bond between gold and sulfur (Au-S) has been widely studied and utilized in many fields. However, detailed investigations on the basic nature of this bond are still lacking. A gold-specific binding protein, GolB, was recently identified, providing a unique opportunity for the study of the Au-S bond at the molecular level. We probed the mechanical strength of the gold-sulfur bond in GolB using single-molecule force spectroscopy. We measured the rupture force of the Au-S bond to be 165 pN, much lower than Au-S bonds measured on different gold surfaces (~1000 pN). We further solved the structures of apo-GolB and Au(I)-GolB complex using X-ray crystallography. These structures showed that the average Au-S bond length in GolB is much longer than the reported average value of Au-S bonds. Our results highlight the dramatic influence of the unique biological environment on the stability and strength of metal coordination bonds in proteins.

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– 6. Protein structure to function –

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ParB spreading and chromosomal DNA condensation in bacterial chromosome partitioning system

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In the bacterial chromosome partitioning system (ParABS), the partition protein (ParB) and its regulatory protein (ParA) act cooperatively through *parS* DNA to facilitate chromosome segregation. ParB oligomer and *parS* interact and form a high-order nucleoprotein which is required for the loading of the structural maintenance of chromosomes proteins onto the chromosome for chromosomal DNA condensation. Spo0J, a member of the ParB superfamily, is an essential component of the ParABS. The binding of *parS* and Spo0J from *Helicobacter pylori* (*HpSpo0J*) was characterized. The crystal structure of *HpSpo0J-parS* complex was determined by Se-MAD method. *HpSpo0J* folds into an elongated structure that includes a flexible N-terminal domain for protein-protein interaction and a conserved DNA-binding domain for *parS* binding. A molecular model for ParB spreading and chromosomal DNA condensation that lead to chromosome segregation is proposed.

P-243

Capturing a biological nanospring in actionM. Synakewicz¹, G. Settanni², M. Rief³, L. Itzhaki¹¹University of Cambridge, UK; ²Johannes Gutenberg University, Germany; ³Technical University of Munich, Germany

Tandem-repeat proteins comprise simple repeating structural motifs whose relative orientation determines their overall solenoid shape. Due to their high tertiary flexibility, repeat proteins can remain structurally intact under mechanical stress before unfolding one repeat at a time at higher forces. More importantly, they are able to refold under force with minimal hysteresis. Using an interdisciplinary approach, we are exploring whether solenoidal repeat proteins are simply a molecular recognition platform or whether their mechanical properties are required to direct function. The HEAT-repeat protein PR65 is the scaffolding subunit of protein phosphatase 2A (PP2A) and is an ideal subject to relate mechanical characteristics to enzymatic output. Using diverse protein engineering strategies guided by computational design, we aim to alter PR65 mechanics by stabilising or de-stabilizing its spring-like nature. To interrogate the mechanics of PR65 and the assembled PP2A heterotrimer, we developed a novel method of DNA-handle attachment for force spectroscopy experiments, which are complemented by functional phosphatase assays. By elucidating repeat protein function from a mechanical perspective, we hope to obtain new insights into key cellular processes and disease pathologies.

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Understanding the molecular mechanisms of anti-CD32b monoclonal antibodiesE. Sutton^{1,3}, R. Bradshaw², C. Orr^{1,3}, B. Frendéus⁴, G. Larsson⁴, I. Teige⁴, I. Tews^{1,3}, J. Essex², M. Cragg¹¹Antibody & Vaccine Group, Cancer Sciences Unit, Faculty of Medicine, University of Southampton, SO16 6YD, UK; ²School of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK; ³Biological Sciences, Institute for Life Sciences, University of Southampton, Southampton, SO17 1BJ, UK; ⁴BioInvent International AB, Sölvegatan 41, 22370 Lund, Sweden

CD32b is the sole inhibitory Fc gamma receptor in humans and has been implicated in autoimmune disease and cancer. A panel of anti-CD32b specific monoclonal antibodies (mAb) have been developed. These mAb bind a similar epitope within the receptor yet have opposing, agonistic (activatory) or antagonist (inhibitory), actions. How these antibodies evoke their opposing effects is currently unclear.

Our current model highlights binding geometry and antibody affinity as key characteristics in determining CD32b activity. Agonist mAb form a compact complex with CD32b, promoting receptor clustering. In contrast, antagonist mAb are higher affinity and form more elongated complexes with CD32b, causing dissociation of receptor micro-clusters and reduced activation.

Bringing together data from small angle X-ray scattering studies and molecular dynamics simulations, alongside that from affinity measurements, binding studies and microscopy experiments, structural models describing how these mAb influence CD32b receptor activity emerge.

P-244

Drosophila nucleoplasmin-like FKBP39 forms a partly disordered homotetramerA. Tarczewska¹, M. Kozłowska¹, D. Bystranowska¹, M. Jakóbb¹, M. Taube², M. Kozak², M. Czarnocki-Cieciura³, A. Ożyhar¹¹Dep. of Biochemistry, Wrocław Univ. of Science and Technology, Wrocław, Poland; ²Dep. of Macromolecular Physics, AMU, Poznań, Poland; ³IBB, PAS, Warszawa, Poland

Nucleoplasmins are nuclear chaperones defined by the presence of a highly conserved N-terminal core domain consisting of a set of five monomers that fit together to form a stable β -propeller structure. Recently, nucleoplasmin-like (NPL) pentameric domain was found in *Drosophila* 39-kDa FK506-binding protein (FKBP39) and other chromatin-associated proteins. Our findings however show that in full-length FKBP39, NPL domain forms non-pentameric complexes. Multi-approach molecular mass analysis of the full-length protein indicated that FKBP39 is a homotetramer. Moreover *in silico* analyses confirmed by biophysical studies had revealed partial disorder in FKBP39. Molecular models reconstructed from small-angle X-ray scattering revealed that the NPL domain forms a stable, tetrameric core and that FK506-binding domains are linked to it by intrinsically disordered, flexible chains that form tentacle-like segments. These findings suggest that the distal regions of the polypeptide chain may influence and determine the quaternary conformation of the nucleoplasmin-like proteins. They provide new insights into the conserved structure of nucleoplasmin core domains and a potential explanation for the importance of the tetrameric structural organization of full-length proteins.

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– 6. Protein structure to function –

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Inter-domain communication in protein through intrinsically disordered region (IDR)S.-I. Tate^{1,2}¹Dept. Mathematical and Life Sciences, Hiroshima University, Japan; ²Research Center for the Mathematics on Chromatin live Dynamics, Hiroshima Univ., Japan

The functionally relevant inter-domain communication between the domains linked by intrinsically disordered region (IDR) was explored by NMR in combination with small angle X-ray scattering (SAXS). Based on the ensemble structure analyses and the numerical simulations to reproduce the chemical shift changes along with the substrate concentration, we have demonstrated how the domains cooperate to enhance the protein function through the substantially dynamic allocation of the domains.

Pin1, a proline cis/trans isomerase, comprises two domains linked by 10-residue IDR; one is the substrate binding domain to recognize pSer/pThr-Pro motif and the other is the enzyme domain that rotates the Pro peptide bond in the motif. The enzyme domain shows very limited affinity to the substrate, but its binding ability was enhanced by two orders of magnitude in the presence of the substrate binding domain linked by IDR, in which the inter-domain ‘fly-casting’ mechanism plays to keep the substrate bound to Pin1: tossing and receiving the substrate between the domains occurs, once the substrate is bound to either one of the domains. A new functional aspect of IDR linking folding domains will be addressed.

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Protein-ligand binding volume determined by fluorescent pressure shift assay, densitometry, and NMRZ. Toleikis¹, V. A. Sirotkin², G. Skvarnavičius¹, J. Smirnoviene¹, C. Roumestand³, D. Matulis¹, V. Petrauskas¹¹Institute of Biotechnology, Vilnius University, Lithuania;²A.M. Butlerov Institute of Chemistry, Kazan Federal University, Russian Federation; ³Centre de Biochimie Structurale, Universités de Montpellier, France

The volume of a protein has many contributing factors arising from the intrinsic volume of the molecule’s atoms, inhomogeneities of protein spatial packing (cavities and clefts), also volume changes related to solvent mediated interactions and thermal fluctuations. In addition to the mentioned factors, protein volume changes can be a consequence of protein-ligand binding. This ligand-induced change in protein volume is referred to as the protein binding volume, which is important but quite largely neglected thermodynamic parameter from the perspective of both fundamental science and potential applications in the development of specific protein ligands. Here we report the values of recombinant human heat shock protein 90 binding volumes, which were obtained by three independent experimental techniques – fluorescent pressure shift assay, vibrating tube densitometry, and high-pressure NMR. Within the error range all techniques provide similar volumetric parameters of investigated protein-ligand systems. Binding volumes of tightly binding, subnanomolar ligands were significantly more negative than those of weakly binding, millimolar ligands.

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A novel, proton-detected, MAS solid-state NMR experiment for the assignment of side chain nucleiJ. Tolchard¹, M. Pandey², M. Berbon¹, A. Noubhani¹, S. Saupe³, Y. Nishiyama², B. Habenstein¹, A. Loquet¹¹Institute of Chemistry & Biology of Membranes & Nanoobjects (UMR5248 CBMN) CNRS, Université de Bordeaux, 33600, Pessac, France; ²JEOL RESONANCE Inc., Musashino, Akishima, Tokyo 196-8558, Japan; ³Institut de Biochimie et de Génétique Cellulaire (UMR 5095 IBGC), CNRS, Université Bordeaux, 33077 Bordeaux, France

In recent years magic-angle spinning (MAS) solid-state NMR has emerged as a broadly applicable biophysical tool for the investigation of biological systems. In particular, improvements in NMR probe construction have enabled faster MAS speeds (>100 kHz) to be attained, which allow for improved spectral resolution and sensitivity. Here, we present a new proton detected 3D MAS experiment for the observation of {H}C-C-H NMR correlations. Our approach is demonstrated on the HET-s prion domain in its functional amyloid fibrillar form. We have acquired an {H}C-C-H experiment at 600 MHz with 70 kHz MAS. Comprehensive agreement can be seen between the measured and previously published ¹³C chemical shifts (Wasmer et al., Science 2008) for the residues of the HET-s rigid core, which allowed us to additionally assign the majority of the H α resonances (97%) as well as protons of the aliphatic and aromatic side chains (69%). The novel approach for the combined identification and assignment of side-chain nuclei highlights its future potential for the study of insoluble and non-crystalline biological systems.

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Novel helical assembly in arginine methyltransferase 8S. Toma-Fukai¹, J.-D. Kim², K.-E. Park³, N. Kuwabara⁴, N. Shimizu⁵, E. Krayukhina⁶, S. Uchiyama⁶, A. Fukamizu^{2,3}, T. Shimizu¹¹Grad. Sch. of Pharm. Sci., The Univ. of Tokyo, Japan;²Life Sci. Center, TARA, Univ. of Tsukuba, Japan; ³Grad.

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Posttranslational modification is a well-known mechanism for regulating molecular interactions. Protein arginine methylation is one type of posttranslational modification. The physiological roles of protein arginine methylation catalyzed by protein arginine methyltransferases (PRMTs) have been established in signal transduction, mRNA splicing, transcriptional control, and so on. Arginine methylation is quantitatively one of the most extensive protein methylation reactions to occur in mammalian cells. PRMT8 is unique among PRMTs, as it is specifically expressed in brain and localized to the plasma membrane. Here, we describe the crystal structure of *human* PRMT8 (*hPRMT8*). The crystal structure of *hPRMT8* exhibited a novel helical assembly. Biochemical, biophysical and mutagenesis experiments demonstrated that *hPRMT8* forms an octamer in solution. This octameric structure is necessary for proper localization to the plasma membrane and efficient methyltransferase activity. The helical assembly might be a relevant quaternary form for *hPRMT1*, which is the predominant PRMT in mammalian cells and most closely related to *hPRMT8*.

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– 6. Protein structure to function –

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HD exchange and MD simulation study - dipeptidyl peptidase III intrinsic dynamics and ligand bindingS. Tomić¹, S. Kazazić¹, M. Abramić¹, M. Tomin¹, D. Agić^{1,2}, Z. Karačić¹, M. Grabar Branilović¹¹Ruder Bošković Institute, Bijenička cesta 54, Zagreb, Croatia; ²Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture, Osijek, Croatia

We employed hydrogen/deuterium exchange reaction combined with molecular dynamics (MD) simulations to investigate conformational dynamics and ligand binding within the M49 enzyme family. Six, two-domain study dipeptidyl peptidase III (DPP III), orthologues, human, yeast, three bacterial and one moss were studied. According to the results all orthologues seems to be quite compact with protected regions located within the two domains core and with the overall flexibility profile consistent with semi-closed conformation as the dominant protein form in solution. Furthermore by comparing HDX data obtained for unliganded protein and its tynorphin complex it was found that tynorphin binds within the inter-domain cleft in all orthologues, but in different orientations. Docking combined with MD simulations revealed details of the protein ligand interactions on human, yeast and three bacterial DPPs III. H-bond analysis revealed that the interdomain active site cleft is more protected in the complexes than in apo enzyme and enabled interpretation of conformational changes noticed at regions distant from the binding site.

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Resolving border conflicts: redefining structural domain boundaries for a biofilm-forming proteinL. van Beek¹, A. Bateman², C. G. Baumann¹, J. R. Potts¹¹Department of Biology, University of York, Wentworth Way, York YO10 5DD, UK; ²European Bioinformatics Institute, Wellcome Genome Campus, Hinxton CB10 1SD, UK

Staphylococcus aureus can cause infections by forming a biofilm on the surface of in-dwelling medical devices¹; such infections are often less susceptible to treatment with antibiotics². Bacterial surface proteins can play an important role in biofilm formation³. For example, *S. aureus* surface protein C (SasC) has been shown to mediate biofilm formation through its N-terminal region⁴. The repetitive central region of SasC contains domains of unknown function (DUF1542). We hypothesise that this repetitive region forms an elongated structure that projects the functional N-terminal domain away from the cell surface.

We proposed new domain boundaries for DUF1542 based on *in silico* analyses. These new domain boundaries were verified by biophysical analyses and ultimately by solving the crystal structure of a double DUF1542 repeat. Knowledge of the structure and function of the repeating domains in surface proteins contributes to a better understanding of the mechanism of biofilm formation.

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P-250

Understanding human DPP III mechanism – an aid in rationalization of the mutants (in)activityS. Tomić¹, B. Kovačević¹, A. Tomić¹, M. Matovina¹, Z. Karačić¹, S. Matić¹, M. Abramić¹, D. Agić²¹Ruder Bošković Institute, Croatia; ²Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture, Croatia

Human dipeptidyl-peptidase III (h.DPP III) is a zinc-exopeptidase that hydrolysis dipeptides from the N-terminus of its substrates. Our study presents the first insight into the reaction mechanism of h.DPP III; determined on the model and real systems. The Glu451-assisted water addition on amide carbon followed by nitrogen inversion are shown to be the rate-determine steps with the activation energies in a good agreement with the experimental results for the Leu-enkephalin hydrolysis. We found that precisely defined geometry of the enzyme binding site puts an additional restraints on the tetrahedral intermediate and stimulates the forward reaction towards final hydrolytic product. Namely, differently from the model, the N-inversion is in concerted fashion followed by favourable hydrogen bonding with Glu451 that immediately “locks” the system into the configuration where reversion to the enzyme-substrate complex is hardly achievable. Therefore we propose that the functional significance of DPP III is dual: to lower the energy barrier of the peptide hydrolysis and to suppress the reverse reaction. Based on the determined mechanism and protein dynamics we were able to rationalize the experimentally determined activity of several h.DPP III mutants detected in human cancers.

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Advanced visualisation and access to PDB data

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Protein Data Bank in Europe, EMBL-EBI, UK

The Protein Data Bank in Europe (PDBe) continually strives to provide accessible, intuitive and effective tools that help the scientific community in understanding and utilizing the rich information content of 3D macromolecular data. In a recent collaboration with CEITEC we have incorporated a highly efficient, in-browser molecular visualizer, LiteMol, which enables users to display structural models, electron density maps of crystal structures and electric potential maps for cryo-EM models in an interactive manner. It also allows overlay of additional layers of information such as domain architecture and validation details.

Keeping up-to-date annotations is essential for providing biological context to the data in the PDB archive. SIFTS (pdbe.org/sifts) [2] provides residue-level mappings to UniProt entries and annotations from a variety of other databases, refreshed on a weekly basis. Additionally, PDBe pages combine data from other relevant resources using a collection of web components which rely on our RESTful API. This API provides convenient programmatic access to data (www.ebi.ac.uk/pdbe/api) and is used in educational services such as Proteopedia and JalView.

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– 6. Protein structure to function –

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Characterization of a bacterial α 2-macroglobulin, a pan-peptidase inhibitor

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α 2-macroglobulins (A2Ms) are proteins which act as broad-spectrum protease inhibitors. Its inhibitory mechanism consists in the capture of the protease followed by the formation of a covalent bond between both proteins. The interaction of the A2M with various classes of proteases might allow its utilization in the identification of proteases in cell extracts, being potentially applied both in the discovery of new proteases or in the detection of a target protease. This study aimed to express, purify and characterize the bacterial A2M (*Salmonella enterica*) for its subsequent use in the identification of proteases. The protein was expressed in *E. coli* BL21 (DE3) and then purified by IMAC and SEC. The secondary structure pattern of A2M in a wide range of pHs, thermal stability and the conformational change were analyzed. The A2M represented secondary structural change over a wide pH range. In addition, protein aggregation during thermal unfolding monitored by circular dichroism indicates that A2M is not thermostable. Moreover, the electrophoretic pattern as well as SEC elution profile indicated a conformational change of the protein upon binding to trypsin. Further investigations are warranted in order to characterize the A2M /Trypsin complex as well as its crystallization.

P-255

Structure and function of highly repetitive bacterial surface proteins involved in infection

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Highly repetitive bacterial surface proteins of Gram-positive bacteria play important roles in infection. We are studying both the functional and structural/biophysical aspects of highly repetitive proteins on the surface of streptococci and staphylococci. We previously showed that the *Staphylococcus aureus* surface protein SasG, involved in inter-bacterial interactions in biofilms, forms a highly elongated, mechanically strong structure on the 70nm scale from a single protein chain (1,2). Here we will also present more recent data including novel domain structures from streptococcal surface proteins. Most repetitive multi-domain proteins have adjacent domains with <40% sequence identity, which is a proposed evolutionary strategy to avoid inter-domain misfolding (3). Thus we are also studying high identity tandem repeats found in some bacterial surface proteins to understand if/how they avoid inter-domain aggregation.

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P-254

Structural Basis for Human Thrombopoietin Receptor Recognition by Janus Kinases

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Thrombopoietin receptor (TpoR) is a type 1 cytokine receptor that regulates the production of blood platelets. Lacking any intrinsic signaling activity, these types of receptors rely on a group of nonreceptor kinases – Janus kinases (JAKs) – to bind their intracellular parts and transduce the cytokine-activated signals. In a pathological state, specific mutations cause this receptor complex to be constitutively active, leading to malignancies, such as familial essential thrombocythemia. Yet, the structure of the interaction complex as well as the mechanisms leading to the autoactivation remain unknown. In this study, we modeled the TpoR–JAK interaction *in silico* with atomistic precision and subsequently probed the dynamics of these structures using atom-scale molecular dynamics simulations. We focused on two main topics. We first studied two types of JAKs (JAK2 and TYK2) and, in both cases, mapped their primary binding sites on TpoR. Second, we unraveled how the TpoR–JAK binding complex anchors itself to the cell membrane. The new knowledge helps us to elucidate the function of JAKs in cytokine-related signaling and provide a platform for further studies on the pathologically relevant mutations.

P-256

Interaction of myelin basic protein with model myelin lipid monolayers at the air-water interface

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To facilitate rapid nerve conduction, a myelin sheath is wrapped around the axons of our neurons. The myelin basic protein (MBP) as one of the major protein components of the central nervous system associates with opposing leaflets of the myelin and thereby ensures a high compaction of the myelin. In case of neurodegenerative diseases like multiple sclerosis a loss of myelin compaction leads to the demyelination of axons and as a result to neurological disorders.

Since the molecular mechanisms of the protein-lipid interaction are still not known, the adsorption of MBP to model lipid monolayers similar to the myelin was studied at the air-water interface. Measuring the surface pressure and the related maximum insertion pressure of the protein for different lipid compositions provides information about the role of the single lipids in the myelin. Especially the interaction of the positive charged MBP with negatively charged lipids (e.g. brain PS) will be examined due to a special adsorption behaviour.

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– 6. Protein structure to function –

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Purification of Ultraspiracle protein from *Helicoverpa armigera* and its structural analysisK. Wycisk¹, M. Kaus-Drobek², A. Ożyhar¹¹Department of Biochemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland; ²Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland

Ultraspiracle (Usp) is a homologue of human nuclear receptor - *retinoid X receptor*. In arthropods Usp controls developmental processes, notably *via* heterodimerization with *ecdysone receptor*. Bioinformatics analyses of the sequence of Usp from *Helicoverpa armigera* revealed that except of two globular domains: DNA and ligand binding, the protein possesses intrinsically disordered regions (IDRs). In contrast to well-known molecular properties of nuclear receptors globular domains not much is known about properties of their IDRs. For that reason we had decided to purify Usp for complex structural analysis. The protein was expressed in BL21(DE3)pLysS *Escherichia coli* cells with expression vector pQE-80L. Usp was purified by metal affinity chromatography and gel filtration. Circular dichroism spectra indicated predominant content of α -helices, however deconvolution of the spectra using CDPro package demonstrated 23% content of disordered regions. The exact position of IDRs was further determined by hydrogen deuterium exchange (HDX). Our results revealed a complex structure of Usp consisting of well-folded DNA and ligand binding domains and disordered N-terminal domain. Together these findings are good basics for further structure-function related studies of IDRs in nuclear receptors.

P-259

Structure and function of endolysin protein of enterococci phage

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Enterococcus (*Enterococcus*) as a common infection of pathogens, usually cause urinary tract infections, abdominal infections, wound infections, bacteremia, mainly seen in patients with low immunity or excessive use of antibiotics, can also lead to sepsis, endocardium Inflammation and other life-threatening, mortality rate of 21.0% to 27.5%. Phage lysis is one of the key enzymes that degrade bacterial cell wall. Typically, a gram-positive bacterial endolysin is a C-terminal region (CWB) having one or two N-terminal catalytic domains (CDs) and responsible for the cell wall, and these two domains are typically composed of two Gene expression to form complexes to function. In the *enterococcus* study, the phage endolysin gene lys170 consists of the full-length endolysin (Lys170FL) and the C-terminal fragment corresponding to the CWB domain (CWB170). These two domains are expressed in the same gene. We have analyzed the tetrameric structure of the CWB domain. If we can analyze the structure of the CWB and CD domain complexes and find the key factors that bind to the bacterial cell wall, this will provide new drug targets for the treatment of enterococci-induced diseases, For the development of drug-resistant *Enterococcus* to lay a solid theoretical foundation.

P-258

A biophysical and biochemical study of SPSB2 and its inhibitory peptides

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The SPRY domain-containing SOCS box proteins (SPSB1 to 4) are a family of four proteins that consist of a central SPRY domain and a C-terminal SOCS box. SPSB1, 2, 4 are negative regulators of inducible nitric oxide synthase (iNOS), which bind to the N-terminus of iNOS via their SPRY domains and target iNOS for ubiquitination and proteasomal degradation [1]. Activated SPSB2-deficient macrophages produced higher level of nitric oxide (NO) and had enhanced *Leishmania* killing compared to control macrophages [1]. Thus, molecules that can inhibit SPSB-iNOS interaction may enhance nitric oxide production and may have potential therapeutic applications in pathogen and cancer cell killing. Here we report the biophysical analysis of a series of SPSB inhibitory peptides using NMR and ITC. We have also solved crystal structures of the SPRY domain of human SPSB2 in complex with inhibitory peptides.

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– 7. Nanobiophysics –

P-260 (O-48)

The mechanical properties of HIV-1 capsid during reverse transcription: insights into uncoating
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For successful infection, the HIV-1 genome, which is in the form of a single-stranded RNA enclosed inside a capsid shell, must be reverse transcribed into double-stranded DNA and released from the capsid (in a process known as uncoating) before it can be integrated into the target cell genome. Although HIV-1 uncoating has been linked to reverse transcription of the viral genome in target cells, the mechanism by which uncoating is initiated is unknown. Using time-lapse atomic force microscopy, we analyzed the structure and physical properties of isolated HIV-1 cores during the course of reverse transcription *in vitro*. We find that, during reverse transcription the pressure inside the capsid increases, reaching a maximum after 7 hours. High-resolution mechanical mapping reveals the formation of a coiled filamentous structure underneath the capsid surface. Subsequently, this coiled structure disappears, the stiffness of the capsid drops precipitously to a value below that of a pre-reverse transcription core, and the cores partially or completely rupture. We propose that the transcription of the relatively flexible ssRNA into the more rigid RNA-DNA hybrid elevates the pressure within the core, which induces uncoating.

P-262 (O-46)

Probing early virus binding steps towards living cells using force-distance curve-based AFM

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Currently, there is a growing need for methods that can quantify and map the molecular interactions of biological samples, both with high-force sensitivity and high spatial resolution. Force-distance (FD) curve-based atomic force microscopy is a valuable tool to simultaneously contour the surface and map the biophysical properties of biological samples at the nanoscale. This presentation reports the use of advanced FD-based technology combined with AFM tips functionalized with single virions to probe the localization of specific binding sites on living cells at high-resolution. We also introduce experimental and theoretical developments that allow force-distance curve-based atomic force microscopy (FD-based AFM) to simultaneously image cell surfaces and to quantify their dynamic binding strength to single virus particle. These binding strengths provide kinetic and thermodynamic parameters of individual ligand-receptor complexes and shed new light into the understanding of how viruses exploit fundamental cellular processes to gain entry to cells and deliver their genetic cargo.

P-261 (O-47)

Using AFM to study red blood cells' morphology and elasticity on Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease. Patients' complications promote changes in hemodynamic properties and abnormalities in red blood cells (RBC) membrane and on its lipid content. Our main goal was to evaluate changes in the viscoelastic and morphological properties of RBCs in ALS. Blood samples from ALS patients were compared with healthy donors. By Atomic Force Microscopy, RBCs' membrane roughness, elasticity and morphological parameters were analysed for both groups. Patients' RBCs are stiffer, have higher penetration depth and are more capable to deform. RBCs from ALS patients are thicker and have a higher cell area. Zeta-potential analysis showed that RBC membranes from ALS patients are less negatively charged, which may be due to a lower density of sialic acid residues on the RBC surface. Patients' RBC membranes have an increased roughness. ALS patients RBC membranes, assessed by fluorescence spectroscopy, are more fluid. This may be associated with changes on membrane lipid composition and packing. We conclude that RBCs from ALS patients present significant electrostatic and morphologic changes on their membranes. These findings may contribute to understand the complex interplay between disease progression rate and RBCs lipid profile.

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The extracellular domain of Her2 receptor: dimerization mechanism and sensitive detection in blood

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The sensitive detection of cancer biomarkers circulating in the bloodstream is timely for early stage disease detection and for developing personalized anti-cancer strategies. In this framework, we optimized surface functionalization protocols and employed novel molecular binders to develop miniaturized platforms for biomarkers detection in body fluids. With the first type of device, based on Atomic Force Microscopy, we optimized biorecognition conditions for best efficiency, then exported to microfluidic implementable platforms based on electrochemical impedance spectroscopy readout. We focused on the detection of the extracellular domain on the Human Epidermal Growth Factor Receptor (Her2), involved in different types of cancer, demonstrating to reach values well below the cut-off limit presently used in clinics for Her2-positive cancer detection, also when Her2 is detected with another biomarkers for cell migration. In parallel we studied the overexpression, dimerization and ECD shedding mechanism in cell membranes, to correlate it with ECD-Her2 levels in the blood and in exosome, nm-sized vesicles involved in distal cell-cell communication, to elucidate the molecular mechanism of Her2 expressing cancer to impact cancer diagnosis, prognosis, and therapy.

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– 7. Nanobiophysics –

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Uni-molecular study of the pH- and salt-dependent PAMAM dendrimers- α -hemolysin nanopore interactionsA. Asandei¹, A. Ciuca², A. Apetrei², I. Schiopu¹, L. Mereuta², C.-H. Seo³, Y. Park⁴, T. Luchian²¹Interdis Re Dept, UAIC, Iasi, Romania; ²Dept Phys, UAIC, Iasi, Romania; ³Dept Bioinfo, Kongju Natl Univ, Kongju, South Korea; ⁴RCPM, Chosun Univ, Gwangju, South Korea

In this work, we investigated the interactions between generation 1 PAMAM dendrimers and the α -hemolysin nanopore, at different pH's and salt concentrations, via single-molecule electrical recordings. Statistical analysis of the ion current recordings through the nanopore during its interaction with the dendrimers demonstrates that net charge changes on the dendrimer and the nanopore alter the kinetics of the dendrimer-nanopore interactions, as well as the extent of nanopore blockade by the dendrimer. Due to the interplay of electrophoretic and electro-osmotic forces acting on the dendrimer, combined with changes in the flexibility of its branches, the dendrimer-nanopore association occurs faster at neutral than acidic pH, while the sojourn of the dendrimer inside the nanopore lasts longer in the former case. We employed a simplified first passage time analysis of the one-dimensional diffusion of the dendrimer through the nanopore, and estimated the diffusion coefficient of the dendrimer inside the confinement of the nanopore at varying pH's and salt conditions. **Acknowledgements:** 2014K1A1A2064460 and 2016R1A2A1A05005440 NRF of Korea, Global Collaborative R&D program (N0001229); 64/01.10.2015 PN-II-RU-TE-2014-4-2388.

P-266

Tailoring protein nanomechanics with chemical reactivityA. E. Beedle¹, M. Mora¹, S. Lynham², G. Stirnemann^{3,4}, S. Garcia-Manyes¹¹Department of Physics, King's College London, UK; ²Centre of Excellence for Mass Spectrometry, King's College London, UK; ³CNRS Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimique, France; ⁴PSL Research University, France

The nanomechanical properties of elastomeric proteins determine the elasticity of a variety of tissues. A widespread natural tactic to regulate protein extensibility lies in the presence of covalent disulfide bonds, which significantly enhance protein stiffness. The prevalent in-vivo strategy to form disulfide bonds requires the presence of dedicated enzymes. Here we propose an alternative chemical route to promote non-enzymatic oxidative protein folding via disulfide isomerization based on naturally occurring small molecules. Using single molecule force-clamp spectroscopy, supported by DFT calculations and mass spectrometry measurements, we demonstrate that subtle changes in the chemical structure of a transient mixed-disulfide intermediate adduct between a protein cysteine and an attacking low molecular-weight thiol have a dramatic effect on the protein's mechanical stability. This approach provides a general tool to rationalize the dynamics of S-thiolation and its role in modulating protein nanomechanics, offering molecular insights on how chemical reactivity regulates protein elasticity.

P-265

Effect of nano zinc oxide crowding on modulation of amyloidogenicity and cytotoxicity of bovine insulinS. Asthana, P. S. Nayak, J. Roy, B. Mallick, S. Jha
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Amyloidosis belongs to a class of disorders caused by protein aggregation and links more than 30 different diseases. Insulin has also been shown to promote injection localized amyloidosis in diabetic patients who rely on insulin injections. Nanomaterials present profound applications in biomedicine due to their immense physico-chemical properties. We synthesized zinc oxide nanoparticles (ZnONP) with negative surface potential and studied its impact on fibrillation and cytotoxicity of bovine insulin at physiological pH. Strong cooperative interaction between insulin and ZnONP was depicted by isothermal titration calorimetry (ITC). The binding and change in conformation of insulin was further analyzed by steady state and time resolved fluorescence spectroscopy. ZnONP drastically enhanced insulin fibrillation and shortened the lag phase as analysed by thioflavin T assay. Morphology of the aggregates were analysed by transmission electron microscopy and increase in the β -sheet content was confirmed by circular dichroism. Additionally, we also demonstrate the cytotoxic propensity of ZnONP assisted insulin amyloidosis on MIN6 cell lines. Thus, our study is a representation of how nanoparticles can have enormous effects on protein misfolding and fibrillation.

P-267

Protein translocation through an α -hemolysin channel: insight from molecular dynamics simulationsE. L. Bonome¹, D. Di Marino², A. Tramontano³, M. Chinappi⁴¹Dipartimento di Ingegneria Meccanica e Aerospaziale Sapienza Università di Roma, Italy; ²Dipartimento di Informatica, Università della Svizzera Italiana, Lugano, Switzerland; ³Dipartimento di Fisica, Sapienza Università di Roma, Italy; ⁴Dipartimento di Ingegneria Industriale, Università di Roma Tor Vergata, Italy

Nanopore sensing is a technique that allows to investigate the conformation and the sequence of macromolecules at single molecule level. When the translocating molecule is a globular protein and the pore narrower section is smaller than the protein size, the translocation is associated to a partial or a complete unfolding of the protein structure.

In this study, we present computational results on the co-translocational unfolding of proteins (Thioredoxin and Ubiquitin) in the α -hemolysin channel. We employed a computational protocol of non-equilibrium all-atom molecular dynamics simulations. The simulations show that both protein translocates in a multistep process where they get stuck in the pore in specific and reproducible conformations (translocation intermediates). In particular, for Thioredoxin, our data indicate that the translocation involves two main barriers and suggest the structures of the two translocation intermediates, providing a reasonable molecular interpretation of the experimental results by Rodriguez-Larrea and Bayley, *Nature nanotechnology* 8.4, 288-295 2013.

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– 7. Nanobiophysics –

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Cell membranes integrity affected by C60 fullerene

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C₆₀ fullerene (FC₆₀) is reported to directly interact with numerous groups of drugs and penetrate into cells. Therefore, it is extensively studied as a drug carrier in novel therapies. However, some reports suggest FC₆₀ toxicity, what might be connected with its hydrophobicity, ability to interact with fatty acids and to affect cellular membranes integrity. From application point of view, it seems important to characterize FC₆₀ interactions with diverse types of membranes.

Three model organisms (Gram+ and Gram- bacteria, yeasts) were chosen to study changes in cell membranes parameters caused by presence of FC₆₀. Using bacterial mutagenicity assay (Ames test) FC₆₀ biological activity was determined. Subsequently, Atomic Force Microscopy was employed to visualize FC₆₀ adhesion to either bacterial or eukaryotic cell walls. Next, combined Live/Dead staining was used to define FC₆₀ uptake and its impact on the cell wall continuity and cells viability. In the last approach, FC₆₀ influence on cell growth rate was assessed by spectrophotometrical measurements.

The obtained findings shed light on FC₆₀ impact on various membranes integrity. Precise understanding of FC₆₀ behavior during movement inward cells is crucial for its future application as a drug transporter in nanomedicine.

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Probing the heterogeneity of protein kinase activation in cells by super-resolution microscopy

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Heterogeneity of mitogen-activated protein kinase (MAPK) activation in genetically identical cells remains poorly understood. MAPK cascades integrate signals emanating from different EGFR spatial locations, including the plasma membrane and endocytic compartment. We hypothesized that in EGF-stimulated cells the MAPK phosphorylation (pMAPK) is largely determined by the spatial organization of EGFR clusters within the cell. We used super-resolution microscopy to resolve the number density of receptors and average intra-cluster distances. Then, we predicted the extent of pMAPK with 85% accuracy on a cell-to-cell basis with control data returning 54% accuracy. Electron microscopy revealed that the most predictive clusters were primarily localized to the limiting membrane of multivesicular bodies (MVB). Tighter packed dimers/multimers were found on intraluminal vesicles within MVBs, where they were unlikely to activate MAPK because of the physical separation. Our results suggest that cell-to-cell differences in the number and localization of receptors can predict EGFR-activated cellular pMAPK levels and account for the heterogeneity in isogenic cells.

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Detecting synthetic sequence-encoded polyphosphates with biological nanopores

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Interaction of polymers with pore-forming membrane proteins may be classified into two types: a binding reaction where the polymer interacts as a particle or the threading of a polymer chain that can allow sequencing. To explore the possibility of sequencing synthetic polymers, we studied the interaction of long, negatively charged polymers with biological nanopores in KCl-solutions ≤ 1M. These polymers, which are sequence-encoded polyphosphates prepared using the phosphoramidite approach on a DNA synthesizer, showed interaction with both α-hemolysin and aerolysin nanopores. We observed interaction with polyphosphate backbones of 56, 64 and 104 monomers. With aerolysin, at 100mV in KCl 300mM, the characteristic dwell times of blocks for these molecules ranged from ~7 to 10ms, whereas with α-hemolysin, blocks lasted for more than a second and were sometimes irreversible. With aerolysin, we also observed interaction of a 64 monomer-long polyphosphate homopolymer and block copolymer bearing alkyne side chains. These interactions showed dwell times at 100mV in 1M KCl solution of ~10ms and shorter dwell times in 300mM KCl of ~1ms. Both types of polyphosphates showed multilevel event current blocks, which might indicate more complex dynamic of interaction in comparison to polynucleotides.

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Understanding Adenovirus maturation: A nano-mechanics approach

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The ability of adenoviruses to infect a broad range of species and tissues has led to a widespread interest in their biological functioning. However, there remains a big gap in our understanding of their assembly and maturation pathways. Here, we present AFM (Atomic Force Microscopy) nanoindentation and fatigue studies^{1,2} of adenovirus capsids³ at different stages of maturation. Surprisingly, we find that the intermediate (no DNA) immature capsid is mechanically indistinguishable as compared with the mature (DNA filled), suggesting a major stabilizing role of the scaffold protein.³ However, these capsids have distinctly different disassembly pathways, as indicated by a mechanically-induced fatigue analysis. Additionally, we observed that mutation of the protease cleavage site of the precursor protein VI yields a maturation-intermediate capsid, G33A, which has reduced infectivity and releases half as many pentons as the WT capsid. The presented results strongly indicate that the reduced infectivity results from a reduction in protein VI exposure, partially inhibiting lysis of the endosome and leading to abortive infection.

¹Roos et al. *Nat Physics* 2010; ²Snijder et al. *Nat Chem* 2013; ³Snijder et al. *J Virology* 2013

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– 7. Nanobiophysics –

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CHO cells on uniform and patterned SLG: adhesion and proliferationA. El Merhie^{1,2}, N. Mishra³, C. Coletti³, A. Diaspro¹, S. Dante¹¹Department of Nanophysics, Istituto Italiano di Tecnologia, Italy; ²DIBRIS, University of Genoa, Italy; ³NEST, Istituto Italiano di Tecnologia, Italy

In the field of biomedicine, single layer graphene (SLG) grown by chemical vapour deposition (CVD) has been witnessing an increasing interest, since it can be easily transferred onto any other substrates and, therefore, exploited for biosensor development. One of the most important aspects to be studied in this regard is cell-material interaction.

With the aim to study how SLG influences cell behaviour, we have compared proliferation curves of Chinese Hamster Ovary (CHO) cells, an adherent cell line, on different substrates. Specifically, we have compared SLG and glass, either coated or not with PDL. The proliferation rate was higher on SLG substrates (either PDL coated or not) with respect to glass. Furthermore, we have patterned SLG substrates by laser micromachining, to obtain geometrically ordered cell growth. Aiming at investigating the response of CHO cells to these interfaces, we stained CHO focal adhesions (FAs) to illustrate how those different adhesion properties translate into different number and shape of focal adhesion complexes. We observed a stellate morphology of cells in contact with SLG with FAs concentrated to the protruding ends of the cells. While cells in contact with the region not covered by SLG appeared more elongated with FAs situated at the extremities.

P-274

Remodeling of lipid rafts by photoswitchable ceramides characterized under high-speed AFMH. G. Franquelim¹, J. A. Frank², D. Trauner², P. Schwille¹¹Max Planck Institute of Biochemistry, Martinsried, Germany; ²Ludwig Maximilians University, Munich, Germany

In our recent publication [1], we described the synthesis and usage of photoswitchable ceramides for the dynamic remodeling of lipid rafts. Here, three photoswitchable ceramides (ACes), which contain an azobenzene photoswitch at the N-acyl chain, were prepared and incorporated into raft-mimicking supported lipid bilayers (SLBs). Using mainly high-speed atomic force microscopy (AFM), we demonstrated that the ACes enabled reversible switching of lipid domains in SLBs. In the trans-configuration, the ACes localized into the liquid-ordered (Lo) phase. Photoisomerization to the cis-form using UV light, triggered a fluidification of the Lo domains, as liquid-disordered (Ld) “lakes” formed within the lipid rafts. Photoisomerization back to the trans-state with blue light, stimulated a rigidification inside the Ld phase, as the formation of small Lo domains was observed. These changes could be repeated over multiple irradiation cycles, enabling a rapid spatiotemporal control of the lipid raft structure with light.

[1] Frank JA, Franquelim HG, Schwille P, Trauner D (2016) Optical control of lipid rafts with photoswitchable ceramides. *J Am Chem Soc*, 138(39): 12981–12986.

P-273

Iron oxide nanozyme: its mechanisms, improvements and applications in nanomedicine

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Since the first finding that Fe₃O₄ nanoparticles with intrinsic peroxidase-like activity (*Nature Nanotech*, 2007), nanozyme is becoming a rapidly emerging field between biology and nanotechnology. Recently, we paid our attention to study the mechanisms and improvements of iron oxide nanozyme (IONzyme) and develop applications in nanomedicine. We found that modification with a single amino acid could enhanced its catalytic efficiency up to 20-fold by mimicking the enzymatic microenvironment of natural peroxidase (*Chem Commun*, 2017). Employing H-ferritin (HF_n) as a template, we biomimetically synthesized a magnetoferritin nanoparticle, which endow the IONzyme with intrinsic tumor targeting property (*Nature Nanotech*, 2012). We further used HF_n as a nanocarrier, which specifically delivers high doses of Dox to kill tumor cells, while exhibiting excellent safety profiles (*PNAS*, 2014). Employing nanozyme activity of IONzyme, we recently developed a novel nanozyme-strip method, which is 100-fold more sensitive than colloidal gold strip, and comparable with ELISA, while it is much faster (within 30 min) and simpler (without need of specialist facilities). The nanozyme-strip provides a valuable simple screening tool for emergency infectious disease diagnosis (*Biosensor Bioelectron*, 2015).

P-275

Lipid flow-assisted selfassembly of functional nanopatterns on gold in rapid dip-pen nanolithography

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We have investigated the molecular directed self-assembly and surface nanochemistry in “crowded space” provided by the lipid matrix. We have found an unusual nanoscale material transport mode, where the low molecular weight compounds (e.g. ω-terminated alkylthiols), which interact weakly with each other but are highly reactive to the gold substrate, flow from the rapidly moving (up to 100 μm/s) scanning probe onto the surface under the same mechanism as the fluid-membrane forming phospholipids. Interestingly, the two types of compounds instantaneously phase-segregate at the scanning probe/gold substrate interface forming stable self-assembled monolayer (SAM) nanopatterns. We have successfully applied lipid-assisted dip-pen nanolithography (DPN) technique to fabricate SAM nanopatterns of basic (e.g. eicosanethiol) and functionalized (e.g. biotinylated and tris-NTA chelator terminated) compounds over centimeter-sized surface areas. Thus, we show that the developed rapid nanopatterning technique is applicable to prototyping of biomolecular nanoarrays and ECM-mimetic structures for single-cell analysis and manipulation.

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– 7. Nanobiophysics –

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Design principles for coiled-coil based materials derived from their molecular mechanical properties

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Coiled-coils (CCs) are abundant folding motifs in many cytoskeletal and extracellular matrix proteins, suggesting that CC structures possess crucial mechanical function. CCs are further used as self-assembling building blocks in synthetic ECM mimics. Despite their wide abundance in Nature and their application potential as mechanical building blocks, surprisingly little is known about their molecular, mechanistic response to an applied force. With the goal of shedding light on the structure-to-mechanics relationship of CCs, we are applying AFM-based single molecule force spectroscopy to obtain information about molecular forces. In particular, we are focusing on the effect of length and pulling geometry. In a first set of experiments, we show that a 4-heptad CC ruptures at a most probable force of >40 pN when loaded in ‘shear’ geometry, while the rupture force for the ‘unzip’ geometry is below the AFM detection limit. In the ‘shear’ geometry we further observe a clear dependence on CC length; a 3-heptad CC ruptures at significantly lower forces of ~ 30 pN. These results aid the future design of novel sequences with mechanical function and yield important new input for the synthesis of CC-based materials with tunable mechanical properties.

P-278

A protein basket for a quantum dot

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The quantum dots (QD) are very potent fluorophores, with a possibility to use them in more active way, e.g. in photodynamic therapy. However, in a living body, QDs may easily get disassembled, releasing toxic ions. One of the reasons of such a process is low stability of outer, organic coat of nanocrystal, composed from several small molecules. The possible answer to this problem is to replace the coat by one single chain of a protein. We already proved that such a substitution is possible, using membrane scaffold protein. Here, we are presenting two new proteins which should be even better in this role. The main requirement was the ability to form a basket-like structure, fitting QD nanometrical size. We used two templates: rich in helices (an RNA binding protein PUF) and rich in beta-structures (leucine-rich repeat domain, LRR). Some modifications were introduced to get more space into a basket. The expressed proteins were able to assembly with QDs, however mostly forming multimers. The reasons of observed situations and further perspective of such proteins use will be discussed. Research supported by National Centre for Research and Development (Lider/012/445/L-4/12/NCBR/2013) and conducted in NanoFun laboratories POIG.02.02.00-00-025/09.

P-277

Experimental study of the polyion potential in polyelectrolyte

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By increasing the polyion concentration c one gets into semidilute regime in which polyion chains form a mesh with the mesh size (distance between the chains) ξ smaller than the polyion length l . Mesh size depends on polyion concentration as $\xi \propto c^{-0.5}$, as established by small angle X-ray scattering (SAXS). Same dependence was observed for the characteristic length L related to the dielectric relaxation in MHz region. This led to the interpretation that counterions relax in the potential valley which extends from polyion to polyion in the mesh – thus it should be of the same size as ξ .

We have to question this interpretation as we found that L depends also on counterion species. We found that L for polyelectrolytes with divalent counterions is 1.3 times larger than for monovalent. Moreover, we find that L is 5 times smaller than ξ .

It is the counterions themselves that rearrange around the polyion and form a potential valley of width L , smaller than ξ . This L does follow the same law as the Debye screening length – dependence on the square root of ionic concentration. Since in polyelectrolytes without added salt the ionic concentration is proportional to polyion monomer concentration, L and ξ follow the same law – while having a different physical origin.

P-279

The folding complexity of TERRA G-quadruplexes unveiled at the single-molecule level

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Guanine-rich sequences, which can be found in telomeres and the regulatory regions of many genes in the human genome, have the potential to self-associate into four-stranded arrangements known as G-quadruplexes (GQs). The presence of these sequences in living cells, along with the fact that their unique conformation provides selective recognition sites for small molecules, have enabled GQs as important drug-design targets for the treatment of various human, including cancer. Here, we report on the unfolding dynamics of short human telomeric RNA (TERRA) molecules at the single-molecule level by optical tweezers. We find that the inherent capacity of single-stranded RNA to self-interact and fold into a condensate blocks the formation of the GQ with a significant probability. In contrast, single-stranded DNA analogue molecules, which were synthesised with the same sequence, did not show this behaviour. The strong conformational competition exhibited by RNA telomeric sequences confers on these short molecules a unique bistable folding stochasticity that is not found in proteins, which usually fold into a unique conformation for fixed external conditions. This knowledge is important to understand G-quadruplex-binding drugs and the mechanical activity of the telomerase.

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– 7. Nanobiophysics –

P-280

Incorporation of influenza surface proteins into nanodiscs

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Influenza virus envelopes harbor the homotrimeric organized spike proteins hemagglutinin (HA) und the tetrameric neuraminidase protein (NA). Both proteins interact with sialic acid (SA) moieties of the glycocalyx of the host cell membrane. While interaction of HA with SA is important for binding of influenza virus to the host cell, NA plays an essential role for release of assembled virus from the host cell by cleaving SA. To study the interaction of HA and NA with SA and respective multivalent inhibitors competing for SA binding sites at single molecule level we aim to reconstitute HA and NA into nanodiscs. To this end, we employed the approach of Killian and co-workers (Dorr et al. 2014), being essentially a detergent free membrane protein solubilization protocol using styrene maleic acid it never has been easier to solubilize and handle membrane proteins. To this end we followed two strategies. The first was to solubilize and reconstitute directly from native viruses. The second strategy was to integrate HA or NA into nanodiscs from eukaryotic cells expressing viral proteins in the plasma membrane. Here we compare both strategies.

P-282

Revealing binding conformations of protein receptor ligand systems and their nano-arrangement by AFM

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The atomic force microscope (AFM) continuously proves to be a versatile tool in biophysical sciences, especially for biomolecular receptor ligand systems. Although requiring increasingly sophisticated experimental methodology and data processing, its scope of applications is broadening steadily: Not only force-dependent unfolding or unbinding kinetics and folding intermediates can be measured. It can also be used to distinguish different binding conformations of single proteins under native conditions. We show detection of a dual binding conformation in a protein receptor-ligand system and its postulated equivalent in a different system. A top of the line and custom built high speed force clamp AFM determines the origin of the systems' peculiar characteristics. As another application, we show nano-arrangement and surface conjugation of single molecules as two approaches: one relies on a force hierarchy in receptor ligand interactions, the other on locally confined enzymatic ligation to surface tethers. We also present a method for data reduction of hundreds of measurements of an unfolding pathway into its representative force-distance curve, a way to provide ease of data handling while respecting the thermodynamic nature of protein unfolding by maintaining reliable statistics.

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Dendrimers efficiency as a drug delivery system for antiepileptic drugs: A biophysical study

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The use of dendrimers (D) as nanotechnology tools in medicine has soared up in recent years. The PANAM D 4 and 4.5 are specially known as efficient drug delivery systems. In this work the use of these D are studied to enhance the solubility and delivery of the antiepileptic drug Carbamazepine (CBZ). The biophysical characterization included time stability, endurance to a lyophilization process, D-CBZ interaction by FTIR, and kinetic release profile of the drug by microdialysis.

The complexes obtained resulted in drug delivery systems were the ratio CBZ: D was 20:1, which presented stability against the lyophilization process, had a controlled drug release, where interaction was mainly based on hydrophobic forces. In addition, the complexes were tested *in vitro* (cell lines Neuro2A), *ex vivo* (human blood cells) and *in vivo* (larvae Zebrafish *D. rerio*). No hemolytic effect was observed in the *ex vivo* model. Contrary to this, there was a toxic effect of the CBZ in both *in vitro* and *in vivo* studies, but not of the D-CBZ complex: the toxic action of the drug was predicted.

The D as a nanovehicle in this study resulted in an efficient nanovehicle to bind CBZ. The potentiality relies on their ability to significantly prevent the toxic effects of the drug in both *in vitro* and *in vivo* models.

P-283

AC electrokinetic immobilisation of nanoobjects as individual singles in regular arrays

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For the investigation of enzymatic action on the single molecule level at still high throughput, immobilisation of the molecules in regular arrays is a useful approach. Here, the suitability of dielectrophoresis (DEP) for such an arrangement as individual singles is investigated. DEP is a phenomenon where forces are exerted on polarisable particles by the action of an inhomogeneous AC electric field. By the right choice of frequency and amplitude the particles are attracted towards the electrodes and can be immobilised without any chemical modifications of the particles or the surfaces. For this purpose, regular arrays of silicon and tungsten nanoelectrodes were developed. They comprise several thousand electrodes with tip diameters of 50 nm and 500 nm, respectively. Polystyrene nanospheres with diameters ranging from 100 nm to 2 µm are immobilised temporarily or permanently by DEP action. Occupation patterns are investigated by fluorescence microscopy and scanning electron microscopy. The number of objects on each electrode is found to be a function of the ratio between particle and tip size. Deterministic immobilisation of nanospheres as individual singles is achieved at electrode diameters being half of the particle size. Further work aims at the immobilisation of single enzyme molecules.

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Lipid affinity to nanoparticles - possible molecular initiating event for nanoparticle toxicity

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Several experiments indicate that nanoparticles can specifically interact with different biological molecules such as protein and lipids. In this work we address the lipid affinity to nanoparticles since it may drive lipid coat/corona formation and possibly initiates nano-toxicity. To measure such a lipid affinity to nanoparticles two different nanoparticle non-specific approaches have been employed to a dispersion of lipid vesicles and nanoparticles. With dynamic light scattering (DLS) we record time-dependent measurements of correlation time. If the latter change with time, lipid-triggered nanoparticles aggregation and nanoparticle-induced vesicle decomposition start. This can be interpreted as high or moderate affinity, respectively. We complement this results with electron paramagnetic resonance (EPR) measurements. Different spin-probes are used to quantify the ordering of lipids in the vesicles and around nanoparticles. The change in lipid ordering depends on the lipid affinity to nanoparticles.

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TB wire, TB extended ladder and RT-TDDFT predict THz oscillations in DNA monomers, dimers, trimersK. Lambropoulos¹, K. Kaklamanis¹, A. Morphis¹, M. Tassi¹, R. Lopp², G. Georgiadis¹, M. Theodorakou¹, M. Chatzileftheriou³, C. Simserides¹¹National and Kapodistrian University of Athens, Department of Physics, Athens, Greece; ²Georg-August-Universität Göttingen, Fakultät für Physik, Göttingen, Germany;³University of Copenhagen, Niels Bohr Institute, Copenhagen, Denmark

We study electron or hole oscillations in B-DNA monomers (one base pair), dimers and trimers. We employ two Tight Binding (TB) approaches: (I) at the base-pair level i.e. a wire model, (II) at the single-base level, i.e. an extended ladder model including diagonal hoppings, and (III) Real-Time Time-Dependent Density Functional Theory (RT-TDDFT). The frequency content of the oscillations is in the THz regime. The amplitude of the oscillations is negligible within monomers, but it is 1 for dimers made of identical monomers and homotrimers and less than 1 in other cases of dimers and trimers. We compare our results from these three methods [1,2,3,4] in terms of frequency content, maximum transfer percentage i.e. amplitude of oscillations and mean probabilities to find the carrier at the sites and find that they are in good agreement. [1] C. Simserides, Chemical Physics 440, 31 (2014). [2] K. Lambropoulos et al., Ann. Phys. (Berlin) 526, 249 (2014). [3] K. Lambropoulos et al., J. Phys.: Condens. Matter 28, 495101 (2016). [4] M. Tassi, A. Morphis, K. Lambropoulos, and C. Simserides, submitted.

P-285

Magnetic (torque) tweezers to probe mechanical properties of dsDNA

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The read-out and processing of highly-packed DNA is regulated by its mechanical properties and interactions with proteins. We use magnetic tweezers (MT) to probe mechanical characteristics of micrometer sized DNA molecules such as the torsional stiffness and the helical twist.

Currently, we are investigating the precise response of DNA to applied forces and torques at varying salt concentrations, using magnetic torque tweezers (MTT). We find a weak dependence on salt for forces < 1 pN, where bending deformations play a crucial role, suggesting a coupling between bending and twisting. In contrast, no dependence on salt is observed for higher forces, which suggests that the intrinsic torsional stiffness of DNA does not depend on ionic strength.

Additionally, we probe the response of dsDNA to an applied number of turns at constant force for increasing temperatures. We experimentally found that the DNA helix unwinds by 0.01 degree/(°C·bp). This unwinding directly affects the torsional stiffness of DNA, leading to smaller values at higher temperatures. However, these data are very preliminary. We plan to understand this behavior by comparison with all-atom molecular dynamics simulations.

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Frequency content of carrier oscillations along B-DNA polymers

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Continuing our work on B-DNA oligomers and polymers [1–5], we will present our new results, on the frequency content of an extra carrier oscillation along periodic, quasi-periodic, fractal and natural B-DNA polymers made of N monomers. We employ two variants of the Tight-Binding approach, a wire model and an extended ladder model including diagonal hoppings. In the former, the site is a monomer i.e. a base pair, while, in the latter, the site is a base. Initially, we focus on the Fourier Spectra of the probabilities to find the extra carrier at each monomer, having placed it at time zero at a specific monomer. We further define some new physical quantities like the weighted mean frequency (WMF) and the total weighted mean frequency (TWMF) and focus on their large N limit. The WMF of the last monomer, having placed the carrier initially at the first monomer, behaves similarly to the fundamental frequency of a fixed-fixed string. The frequency content is, generally, in the THz domain.

[1] K. Lambropoulos et al., Phys. Rev. E 94 (2016) 062403

[2] K. Lambropoulos et al., J. Phys.: Condens. Matter 28 (2016) 495101

[3] K. Lambropoulos et al., Phys. Rev. E 92 (2015) 032725

[4] K. Lambropoulos et al., Annalen der Physik (Berlin) 526 (2014) 249

[5] C. Simserides, Chemical Physics 440 (2014) 31

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AC electrokinetic manipulation of nanoparticles and moleculesE.-M. Laux¹, X. Knigge^{1,2}, C. Wenger³, F. F. Bier^{1,2}, R. Hölzel¹¹Fraunhofer Institute IZI-BB, Branch Bioanalysis and Bioprocesses, Potsdam-Golm, Germany; ²University of Potsdam, Germany; ³IHP GmbH - Leibniz Institute for Innovative Microelectronics, Frankfurt (Oder), Germany

AC electric fields are increasingly exploited for the separation of nanoparticles and molecules in micro- and nano-electrode systems. AC electrokinetic phenomena like dielectrophoresis (DEP) and AC electroosmosis allow for a robust non-contact spatial manipulation. Here we present results of dielectrophoretic experiments on nanoparticles and proteins in various electrode systems. Numerous protein species have been successfully manipulated: intrinsically fluorescing proteins like eGFP, DsRed and RPE, and fluorescently labeled BSA, antibodies and enzymes. Selective binding of antibodies as well as enzymatic function are shown to be retained after DEP immobilisation. Depending on electrode size, molecules and nanoparticles are isolated as singles between planar triangular electrodes or on arrays comprising up to 500,000 conical electrodes. The factors that influence the particles' distribution are discussed. Using fluorescence polarisation microscopy, permanent dielectrophoretic immobilisation of eGFP molecules is demonstrated with parallel alignment of the proteins' main axis along the electric field, and the relative orientation of the molecules' fluorophore is determined.

P-290

The influence of disulfide bonds in the mechanical stability of proteins is context dependentA. Manteca¹, A. Alonso-Caballero¹, M. Fertin¹, S. Poly¹, D. de Sancho^{1,2}, R. Perez-Jimenez^{1,2}¹CIC nanoGUNE, Spain; ²Ikerbasque, Spain

Disulfide bonds play a crucial role in proteins, modulating their stability and constraining their conformational dynamics. A particularly important case is that of proteins that withstand force in their normal biological function, which are often disulfide bonded. However, the influence of disulfides in the overall mechanical stability of proteins is poorly understood. Here we use single-molecule force spectroscopy (smFS) to study the role of disulfide bonds in different mechanical proteins in terms of their unfolding forces. For this purpose, we have chosen a disulfide-bonded variant of the I91 human cardiac titin and the Gram-negative bacterial protein FimG. The results reveal that disulfide bonds can either enhance or reduce the total mechanical strength. Specifically, disulfide-bonded titin Ig domains suffer a ~13% decrease in their mechanical stability compared to the reduced ones, while the unfolding force of FimG increases by a 26% on its oxidized state. Using a coarse-grained simulation model we rationalize that the increase in mechanical stability of FimG is due to a shift in the mechanical unfolding pathway. However a simple topology-based explanation is not sufficient to explain the effects in titin, suggesting that higher order effects may also be at play.

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Fluorescent labelling of nanoparticles for reliable bio-nano interactions studyH. Majaron¹, B. Kokot¹, I. Urbančič^{1,2}, P. Umek¹, T. Koklič¹, M. Garvas¹, J. Štrancar¹¹Laboratory of Biophysics, Condensed Matter Physics Department, J. Stefan Institute, Ljubljana, Slovenia, Slovenia; ²MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, UK

Fluorescent studies of nanoparticle interactions with biological matter significantly depend on the stability and efficiency of fluorescent labelling of the nanoparticles. With the native nanoparticle surface being unsuitable for direct labelling with most commercial fluorophores, such labelling either requires nanoparticle surface functionalization prior to labelling, or alternatively doping the nanoparticles with fluorescent atoms during the nanoparticle synthesis. In the former case, surface-bound fluorophores provide additional spectrally-coded information about their local molecular environment. However, during the in vitro/in vivo experiments such probes may desorb from the nanoparticles or may be digested by enzymes, making the interpretation of the experiments unreliable. On the other hand, fluorescent lanthanide-doped nanoparticles provide a much more stable alternative, but require doping during synthesis of the original nanoparticles. Moreover, they cannot provide additional information about the local molecular environment. More advantages and pitfalls will be presented in detail for both labelling options - for fluorescently labelled and for europium-doped TiO₂ nanotubes.

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Study of the protein corona formed by the adsorption of hemoproteins on silica nanoparticlesL. Marichal^{1,2}, J.-P. Renault¹, F. Cousin³, S. Combet³, A. Gatin¹, J.-C. Aude², Y. Boulard², J. Labarre², S. Pin¹¹LIONS, NIMBE, CEA, CNRS, Université Paris-Saclay, CEA Saclay, France; ²I2BC, IBITEC-S, CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, France; ³Laboratoire Léon-Brillouin, CEA-Saclay, France

The performance of a bio-nanomaterial essentially results from interactions that occur at its surface and that depend on the adsorption of biomolecules, particularly proteins. In order to improve the biocompatibility of nanomaterials, one of the challenges is to understand the mechanisms of protein adsorption and to appreciate the modifications of adsorbed proteins.

Using model hemoproteins and monodisperse silica nanoparticles (SiNPs) we studied the impact of the protein size on its ability to interact on the silica surface in a qualitative and quantitative way. Adsorption differences between myoglobin and hemoglobin (Devineau *et al.*, Langmuir, 2017) were previously investigated and we decided to extend the comparison to even larger hemoproteins that can be regarded as oligomeric hemoglobins. We studied the hemoprotein/SiNPs interactions using a panel of methods: adsorption isotherms, circular dichroism, small angle neutron scattering, and isothermal titration calorimetry. The end result of this study is that the protein size can have a major impact in terms of global affinity towards a silica surface but also in terms of protein structure alteration and thermodynamic changes caused by the adsorption.

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– 7. Nanobiophysics –

P-292

Quantification of surface binding by wide-field total internal reflection FCSJ. Mücksch¹, P. Blumhardt¹, M. Strauss^{1,2}, R. Jungmann^{1,2}, P. Schwill¹¹Max Planck Institute of Biochemistry, Martinsried, Germany; ²Ludwig Maximilian University, Munich, Germany

The accurate determination of binding rates to membranes or membrane proteins is of key relevance for quantitative biology. Despite the existence of multiple methods to characterize surface interactions, there are still experimental challenges regarding simplicity of use and general applicability. Here, we present a simple versatile single-molecule fluorescence approach for the accurate determination of binding rates to surfaces or surface bound receptors. We combine Fluorescence Correlation Spectroscopy (FCS) with Total Internal Reflection (TIR) Fluorescence microscopy and a camera-based detection. Our approach not only yields a high surface selectivity, but also resolves association and dissociation rates over a wide time range. In a proof of principle application, we quantified the transient hybridization of single-stranded DNA to the complementary handles of immobilized DNA origamis. We varied the nucleotide overlap, yielding different transient binding times in the range of milliseconds to tens of seconds. Using this highly tunable system, we systematically explored the parameter space accessible to EMCCD-based TIR-FCS. Our current focus is to transfer this assay to the otherwise challenging quantification of protein-bilayer and membrane-related protein-protein interactions.

P-294

Biophysical analysis of extracellular vesiclesP. Parisse¹, E. Ambrosetti^{2,1}, P. Paoletti⁴, D. Cesselli³, A. P. Beltrami³, L. Casalis¹¹Elettra Sincrotrone Trieste, Italy; ²Università degli studi di Trieste, Italy; ³Università degli studi di Udine, Italy; ⁴Scuola Internazionale Superiore di Studi Avanzati, Italy

Extracellular vesicles (EVs) are small vesicles ensuring transport of molecules between cells and throughout the body. EVs contain cell-type specific signatures and have been proposed as biomarkers in a variety of diseases. Their small size and biological and physical functions make them optimal candidates for therapeutic agents in immune therapy, vaccination, regenerative medicine, and drug delivery. Indeed there is no objective set of criteria available for designing synthetic EVs for a specific task in biomedicine. It is therefore urgent and critical to address these issues for EVs-based medicine to fulfill its promise. Here, we try to assess the phenotypic properties of EVs, through a multi-technique characterization based on Dynamic Light Scattering (DLS), FTIR spectroscopy, Atomic Force Microscopy (TEM) and Small Angle X-Ray Scattering (SAXS). This detailed analysis allows us to model, visualize and quantify EVs' physical and chemical properties up to single vesicle level and it serves as a basis for the correlation of phenotypic parameters of EVs with their functional activity. The proposed work could allow for the design of innovative strategies for their sorting and detection, and for the personalized nanomedicine in general.

P-293

Quantitative detection of local molecular forces at biological membranesR. D. Ortuso, C. Girard-Reydet, K. Sugihara
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Forces applied on cell membranes are the key activator for touching sensation, immune systems, and tissue growth. Therefore, their quantification is an urgent issue for understanding these phenomena and the mechanism of the related diseases. However, to date there is no direct way to measure the local molecular forces in cell membranes.

My group is developing a fluorescence probe for quantifying local peptide forces in lipid bilayers.[1,2] The probe is based on a mechanochromic polymer, polydiacetylene (PDA). Polydiacetylene emits fluorescence when external forces alter the conformation of the polymer. It is an amphiphilic molecule, thus can be easily incorporated in biological membranes. It is known to react upon peptide binding, but has never been calibrated against local forces due to a lack of technique. We overcame this challenge by introducing a unique characterization tool, friction force microscopy. The obtained calibration curve (fluorescence intensity vs local forces) allows us to use polydiacetylene as a quantitative force sensor for measuring biomolecular forces in bilayers.

[1]C. Girard-Reydet, R. D. Ortuso, M. Tsemperouli, and K. Sugihara, *J Phys Chem B* **120**, 3511 (2016).[2]R. D. Ortuso, U. Cataldi, and K. Sugihara, *Soft Matter* **13**, 1728 (2017).

P-295

Membrane-cytoskeleton bonds rupture in a catch-slip manner at the edge of a cancer cellV. Rajeskhara¹, V. K. Sreenivasan¹, J. N. Myers², F. A. Pereira¹, B. Farrell¹¹Baylor College of Medicine Houston, TX 77003, USA; ²MD Anderson Cancer Center Houston, TX 77003, USA

Cancer cells are capable of cytoskeleton remodeling and membrane reorganization in response to environmental cues. The propensity to remodel in response to a mechanical cue is reflected in part by the lifetime of the membrane-cytoskeleton bonds that can be measured by application of a tensile load. Lifetime of bonds depends upon their intrinsic properties increasing with tensile load when they behave like catch bonds, and decreasing with load when they exhibit slip-like behavior. The *catch-slip* transition between the two pathways indicates a conformational change within the assembly and was reported upon separating isolated protein assemblies *in vitro*. We now report detection of this transition upon peeling the plasma membrane from the cytoskeleton at the top edge of a cancer cell. Experiments were conducted at the near-equilibrium region where an optical tweezers is used to apply a load with a handle which is bound to the slowly moving cell, and the displacement of the handle is detected at a resolution of 0.5 ms after averaging. The force to rupture and lifetime increase concurrently with the loading rate to a maximum value, after which both decrease with increasing loading rate. The characteristics of these bonds, and their response to drug treatments will be presented.

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– 7. Nanobiophysics –

P-296

Cation-induced stabilization and destabilization of DNA origami nanostructures in urea and guanidinium chlorideS. Ramakrishnan¹, G. Krainer², M. Schlierf², G. Grundmeier¹, A. Keller¹¹Department of Chemistry, University of Paderborn, Paderborn, Germany; ²B CUBE – Center for Molecular Bioengineering, TU Dresden, Dresden, Germany

DNA origami [1] is an emerging molecular tool with great potential for many applications in nanoscience, structural biology and biophysics. Precise addressability of staples enables the DNA origami to be decorated with functional organic, inorganic materials and proteins in 2D and 3D arrays with few nanometers resolution which is specifically interesting to study the structural dynamics of biomolecules and intermolecular interactions. The required structural integrity of the DNA origami may, however, pose significant limitations regarding their applicability, for instance in protein folding studies that require strongly denaturing conditions. In our previous work, we have investigated the denaturation of DNA origami by the chaotropes urea and guanidinium chloride in dependence of temperature [2]. Here we report on the effect of cations on the stabilization and destabilization of DNA origami structures in the presence of both agents in dependence of cation species and concentration. In urea, we observe a gradual stabilization of DNA origami as the cation concentration increases whereas in guanidinium chloride, cations are found to promote DNA origami denaturation at high concentrations.

[1] Rothmund et al. Nature (2006) 440, 297.

[2] Ramakrishnan et al. Nanoscale (2016) 8,10398.

P-298

Non-radiative excitation fluorescence microscopy for studying membrane adhesion at the nanoscale

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Non-Radiative Excitation Fluorescence Microscopy (NEFM) [1] constitutes a new way to observe biological samples beyond the diffraction limit. By coating a substrate with an homogeneous monolayer of quantum dots (QDs), Förster Resonance Energy Transfer (FRET) could be achieved between the QDs layer (which play the role of the donor) and Giant Unilamellar Vesicles (GUVs) labelled with DiD (which play the role of the acceptor). The dyes were not directly excited by the laser source but through a non-radiative energy transfer. GUVs were added on a QD layer coated with poly-L-lysine (electrostatic attraction occurred between the positively charged surface and negatively charged GUVs). On this kind of sample, we were able to observe at the same time the emission of the DiD and the quenching of the QDs. It clearly indicates that non-radiative energy transfer occurs from the QDs to DiD. From these two pictures, we also calculated the distance between the lipid membrane and the surface for each pixel with a nanometric resolution.

[1] P. Winckler, R. Jaffiol, J. Plain, and P. Royer. “Nanoradiative Excitation Fluorescence: Probing Volumes Down to the Attoliter Range.” The Journal of Physical Chemistry Letters 1, 2451-2454 (2010).

P-297

Analysis of the mechanical properties of HIV-1 capsid and their impact on the uncoating processS. S. Rankovic¹, R. Ramalho¹, C. Aiken², I. Rouso¹¹Department of Physiology and Cell Biology, Ben-Gurion University of the Negev, Beer-Sheva, 84105, Israel;²Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, USA

The human immunodeficiency virus (HIV-1) core consists of a protein shell called capsid that encapsulates the viral genome. After a viral particle enters a host cell and prior to nuclear import of viral dsDNA, the capsid has to disassemble in a process called uncoating. Several studies have shown that uncoating is promoted by reverse transcription (RT) and regulated by cellular factors. Mutations that alter the stability of the capsid affect the uncoating rate and impair HIV-1 infectivity. Our AFM measurements of the mechanical properties of wild type (WT) and hyperstable mutant cores (HMC) show that mutations that increase stability and delay uncoating also increase the stiffness of the HIV-1 capsid. In addition, we analyzed the morphological and stiffness changes of WT and hyperstable cores during reverse transcription using time-lapse AFM. We find that hyperstable cores remained intact whereas WT cores disassembled. Based on our findings we propose that the mechanical properties of the HIV-1 capsid must be well balanced to enable capsid uncoating followed by infection.

P-299

Structure and dynamics of amylin-lipid mixed fibers from advanced fluorescence microscopyJ. C. Ricardo¹, A. Fedorov¹, F. Fernandes¹, M. Prieto¹, A. I. A. Coutinho^{1,2}¹Centro de Química-Física Molecular and Institute of Nanoscience and Nanotechnology, IST UL, Portugal; ²Dep. Química e Bioquímica, Fac. Ciências UL, Portugal

Biological membranes play an important role in amyloid fibril formation *in vivo* by inducing the misfolding and self-assembly of several amyloidogenic proteins/peptides. In addition they can also promote the formation of supramolecular structures composed of both peptides and lipids. We addressed this topic by studying the interaction of monomeric amylin (IAPP; diabetes type II) with POPS vesicles. The extent of interaction was determined from fluorescence anisotropy data. The structure and dynamics of the amyloid-like mixed fibers were studied at the molecular level using FRET. IAPP was labelled with the donor Atto488 and the acceptor is the fluorescently-labelled lipid Rho-DOPE. We concluded from time-resolved FRET measurements performed both at single fiber (FLIM-FRET) and macroscopic (bulk) level that the mixed fibers display a multilayer structure. FRAP and 2PE Laurdan generalized polarization measurements revealed that these fibers are rigid and present an extensive membrane surface dehydration. We are currently performing fluorescence anisotropy imaging microscopy measurements to further characterize the peptide dynamics in the mixed fibers. Supported by FCT/Portugal' project FAPESP/20107/2014 and grants SFRH/BD/95856/2013 (JCR) and IF/00386/2015 (FF).

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– 7. Nanobiophysics –

P-300

Tuning rhodamine structure for efficient blocking of the alpha-hemolysin nanoporeT. I. Rokitskaya¹, P. A. Nazarov¹, A. V. Golovin², Y. N. Antonenko¹¹Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Russian Federation;²Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Russian Federation

Measurements of ion conductance through α -hemolysin pore in a bilayer lipid membrane revealed blocking of the ion channel by a series of rhodamine 19 and rhodamine B esters. The longest dwell closed time of the blocking was observed with rhodamine 19 butyl ester (C4R1), while the octyl ester (C8R1) was of poor effect. Voltage asymmetry in the binding kinetics indicated that rhodamine derivatives bound to the stem part of the aqueous pore lumen. The binding frequency was proportional to a quadratic function of rhodamine concentrations, thereby showing that the dominant binding species were rhodamine dimers. Two levels of the pore conductance and two dwell closed times of the pore were found. The dwell closed times lengthened, as the voltage increased, suggesting impermeability of the channel for the ligands. Molecular docking analysis revealed two distinct binding sites within the *lumen* of the *stem* of the α -hemolysin pore for the C4R1 dimer, but only one binding site for the C8R1 dimer. The blocking of α -hemolysin nanopore by rhodamines could be utilized in DNA sequencing as additional optical sensing owing to bright fluorescence of rhodamines if used for DNA labeling.

P-302

Surface charge regulated adsorption of semiconductor polymer coated nanorods on lipid membranesB. Salis^{1,2}, A. Quarta³, T. Pellegrino¹, A. Diaspro¹, S. Dante¹¹Istituto Italiano di Tecnologia, Genoa, Italy; ²DIBRIS, University of Genoa, Italy; ³Institute of nanotechnology c/o Campus Ecotekne, Lecce, Italy

The interplay between Nanoparticles (NPs) and cell membranes is the basis of their possible usage for therapeutics. In our group we are currently investigating how NPs of different shape, size and charge interact with neural networks and, in particular, how NPs surface charge is influencing electric activity of neurons. With the aim to model the mechanism at the molecular scale, we studied here the interaction between semiconductor NanoRods (NRs) and model membranes. NRs were in-house synthesized and on purpose functionalized with an amphiphilic polymer to tune their charge between -30 mV and +10 mV. At least three different charges per NRs of equal size and material were obtained. The interaction with lipid mixtures of different complexity was tested. In particular, NRs adsorption to Supported Lipid Bilayers (SLBs) was monitored by QCM-D; the interaction with lipid monolayers was measured with Langmuir isotherms correlated with surface potential measurements. Preliminary results showed that tuning mutual properties of the system regulates the adsorption of NRs on the membranes.

P-301

Single molecule study of DNA phase transitions under forces. A focus on the mixed phase conditionD. Salerno, M. Cristofalo, C. Marrano, E. Chiodi, N. Misana, R. Corti, L. Nardo, V. Cassina, F. Mantegazza
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The DNA molecule, typically found in B-structure, during its physiological activity explores different forms. Its structural transitions are induced by external agents (e.g. temperature modifications, proteins activity).

Recently, the capability of applying forces and torques at the single molecule level allowed the study of the mechanical induced transitions by tuning the external stresses applied to the DNA. For example, the phase diagram of the DNA recently reported is composed by 10 different phases, some of them compatible with physiological conditions and others existing under external stress too far from them.

Even if the most common way to found different DNA phases in cells is in coexistence of different mixed structures, up to now, almost all studies focused on pure DNA phases. In this work we study the DNA structural transitions between B-DNA and the so called left hand DNA (L-DNA), a melted structure generated at negative supercoiling stretched by a sufficient force. Unexpectedly, we discovered that the minimum force value needed to induce a transition increases with the imposed supercoiling, suggesting the dependence of the energetic balance between B and L-DNA on the relative fraction of the two phases, as confirmed by the developed empirical model.

P-303

New X-ray single molecular observations from super-Poisson distribution using laboratory X-raysY. C. Sasaki^{1,2}, K. Ikezaki¹, K. Mio², H. Sekiguchi³¹The University of Tokyo, Japan; ²Operando-OIL AIST/UT, Japan; ³Spring-8/JASRI, Japan

In X-rays, the relationship between intensity and integrated time can be described by the Poisson process. In order to narrow the standard deviation of the X-ray intensity, the integration time is made longer. However, when the nano-size X-ray source moves spatially on a certain time scale, this Poisson distribution is not accurate. In 1998, we proposed a method to observe intramolecular motions by labeling gold nanocrystals with individual single protein molecules and observing the motions of diffracted X-ray spots from labeled individual nanocrystals. Until now, we have observed individual DNA molecules, protein membranes (Bacteriorhodopsin, KcsA, AChBP, nAChR etc.), denatured/unfolded proteins, and major histocompatibility complex. This DXT can trace all motions within single protein molecule using white X-rays. When using monochromatic X-rays, it is impossible to track all movements of diffraction spots, but the diffracted X-ray intensity distribution from the labeled and moving gold nanocrystals correlated with the velocity of the diffraction spots. The intensity fluctuation of the slightly moving diffracted X-rays was larger than that when almost stopped. Here, we will call this the Poisson-shift. This work demonstrated that using this Poisson-shift, we could observe the motions of a single protein molecule even in laboratory X-ray sources.

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– 7. Nanobiophysics –

P-304

Trapping of a single peptide near a protein nanoporeI. Schiopu¹, A. Asandei¹, M. Chinappi², C.-H. Seo³, Y. Park⁴, T. Luchian^{5,1}¹Dept. of Interdisciplinary Research, A.I.I.Cuza University of Iasi, Romania; ²Ctr. Life Nano Sci., IIT, Rome, Italy; ³Dept. Bioinfo., Kongju National University, Kongju, South Korea; ⁴RCPM, Chosun University, Gwangju, South Korea; ⁵Dept. Phys., A.I.I.Cuza University of Iasi, Romania

Nanopore technology is emerging as a relevant tool that enables the research of a variety of features and properties of single peptides, or protein molecules. In this study, we demonstrated the formation of a molecular trap near a wild-type α -hemolysin (α -HL) protein nanopore in an acidic electrolyte solution with the help of the electroosmotic flow. By acting against the electrophoretic force, we showed that the electroosmotic flow allows the peptide to be recaptured continuously inside the protein nanopore. The trapped peptide enters the nanopore, where it can juggle between the two main topologically distinct regions of the α -HL (i.e., the vestibule and lumen), before escaping through either of the nanopore's openings. The statistical analysis of all the possible and observable steps of the peptide's journey inside the nanopore enables the precise identification of the distinct regions in which the peptide resides, and allows a detailed picture of the kinetic mechanism and a thermodynamic description of the peptide reversible binding to and within the nanopore. *Acknowledgements:* PN-II-RU-TE-2014-4-2388; GRL/NRF-2014K1A1A2064460; PN-II-PT-PCCA-2011-3.1-0402.

P-306

Tuning polymer-protein interaction with saltM. Talarimoghari¹, A. Dylewska¹, M. Hoffmann¹, G. Baaken², D. Chouikhi³, J.-F. Lutz³, J. C. Behrends^{1,2}¹University of Freiburg, Germany; ²Physiology, Ionera Technologies GmbH, Freiburg, Germany; ³Institut Charles Sadron, University of Strasbourg, France

Biological nanopores are known to interact with synthetic and biological polymers, enabling their use in label-free single-molecule analytical tasks such as sequencing and/or mass discrimination. Here, we report on polydimethyl acrylamide (PDMAA), a water-soluble neutral polymer. We obtained current blocks with poly disperse PDMAA (PDI=1.08) that were low in frequency ($<1\text{Hz}/\mu\text{M}$) short ($\tau < 100\mu\text{s}$) and noisy, resulting in little mass resolution compared to PEG. We reasoned that we might take advantage of a specific salt effect of fluoride anion reported for the α HL pore in order to increase blocking frequency and dwell time. Using electrolyte solutions consisting of 4M K⁺ as cation and various proportions of Cl⁻ and F⁻ as anions. We were able to obtain longer events (τ up to 225 μs) and higher frequencies (up to 1.42Hz/ μM), allowing significantly better mass resolution for PDMAA than in 4M KCl. However, the large noise component in the blocked current levels for PDMAA as opposed to PEG remained, still compromising the peak-to-valley ratio of histograms. These findings suggest that the specific salt effect of F⁻ on polymer-protein interaction is independent of the polymer and may be useful in tuning polymer pore interaction for a range of analytes.

P-305

Albumin – based γ irradiated nanoparticle: Characterisation, Stability and Binding efficiency

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A bovine serum albumin-based nanoparticle (BSA NP) was achieved by γ -irradiation in an ethanol solution. Its structure and function characterisation of the nanoparticle (NP) is presented.

The NP as a spherical entity was stated by DLS and microscopy techniques. Preservation of the main structural features (α -helix content and main binding pocket) was displayed by: UV-vis, FTIR, Fluorescence, and CD. Surface free SH, NH and CO groups were determined to elucidate the NP's possible interactions with other substances. Moreover, the NP was stable against adverse conditions like pH and chaotropic agents. Results indicated that the NP has a compact structure which prevents denaturation.

The interaction of the NP with the hydrophobic drug Emodin (E) was studied, resulting in a drug -delivery system (NP-E) with anti-tumour potential. The binding and kinetic properties of the obtained complex were tested by biophysical methods as well as its toxicity in tumour cells. Contrary to the NP alone, the NP-E showed a cytotoxic effect on cancer cell lines. No haemolytic activity was found when tested *ex vivo* in red blood cells.

Data obtained describe the BSA NP as a nontoxic and stable entity. The NP preserves the main function of albumin and characteristics of an excellent carrier of molecules.

P-307

Fluorescence/absorbance spectroscopy on dyes absorbed on hybrids of DNA and carbon nanotubesA. Tomura¹, K. Umemura²¹Department of Physics, Faculty of Science Division II, Tokyo University of Science, Japan; ²Dpartment of Physics, Graduate school of Science, Tokyo University of Science, Japan

We conducted spectroscopy to evaluate the interaction between near-infrared (NIR) or visible dyes and hybrids of DNA and single-walled carbon nanotubes (DNA-SWCNTs). These hybrids are expected to work as scaffolds for dye molecules in solutions because SWCNTs are well dispersed with DNA molecules. We used three different dyes; uranine as a visible dye and indocyanine green (ICG) and rhodamine 800 (R800) as NIR dyes (emission wavelengths of each dye are 515, 815 and 710 nm, respectively). Dye solutions were simply added to various concentrations of DNA-SWCNT suspension, and then, fluorescence and absorbance spectra were measured. As a result, fluorescence intensities of uranine, ICG and R800 decreased by the maximum of approximately 40, 70 and 90 % respectively, as the concentration of suspension increased. This indicates the well-known quenching phenomenon caused by SWCNTs. Furthermore, while absorbance spectra of visible dye did not change, those of NIR dyes decreased and the form of spectra changed, suggesting a certain change of dye molecules. Our results provide fundamental information about the interaction between dyes and DNA-SWCNTs.

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– 7. Nanobiophysics –

P-308

Interaction of positively charged Co-porphyrins with liposomes made of a mixture of POPC and POPG
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The adsorption of cationic Co-containing meso-tetra-[4N-oxyethylpyridyl] porphyrins (CoTOEtPyP4) onto the surface of negatively charged liposomes was studied. Liposomes were made of a mixture of POPC and POPG in ratios of 6:4, 7:3 and 9:1. The mobility and size of liposomes were examined by dynamic light scattering. The NaCl concentration dependence of the zeta-potential of liposomes was studied both in the absence and presence of Co-porphyrins. As expected, the zeta-potential in the absence of the porphyrins was found to be negative. It was shown that in high concentrations of cationic CoTOEtPyP4 porphyrin the negative zeta-potential of liposomes changes to a positive one, which indicates that Co-porphyrins adsorb well on the surface of liposomes. At salt concentrations higher than about 40 mM the zeta-potential in the presence of porphyrins gradually decreases becoming lower than its value in the absence of porphyrins because of the interaction of phosphate groups of lipids and amine groups of porphyrins. A theoretical model was developed to describe the obtained experimental data. By fitting the model parameters we could determine binding constants of Na⁺ ions and CoTOEtPyP4 porphyrins to liposomes that are in agreement with the experimental data.

P-310

Switchable assembly of bacteriophage fibres for nanoscale bioengineering using H-aggregate formation

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There are a number of challenges associated with the ordered assembly of molecules and a particular interest in the assembly of fluorescent dyes, chiefly due to the interesting alterations in their optical properties afforded by specific molecular arrangements. The first challenge is self-assembly: how can dyes be made to spontaneously organise? The second challenge is solvent conditions: how can less organic solvent be used when most known fluorescent dyes do not assemble in an ordered fashion in aqueous conditions? The third challenge is control: can the organisation of fluorescent dyes be induced on demand? It is shown here using spectroscopic techniques that by covalently binding tetramethylrhodamine (TRITC), a fluorescent dye that forms H-aggregates, to the surface of M13 bacteriophage, the ordered self-assembly of TRITC can be greatly increased in aqueous conditions. Additionally it is shown that the assembly of the M13TRITC conjugate can be controlled by the addition of ammonium sulphate. Following this demonstration it is hypothesised that M13 could be used to promote the self-assembly of dyes that form J-aggregates, a rare arrangement of fluorescent dye, which has interesting optical properties and potential applications in the field of light harvesting technology.

P-309

Alkali cations modify the stiffness of biomembranes by forming slowly evolving interfacial networks

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Simple alkali cations such as sodium and potassium are abundant in aqueous systems, especially biological organisms, where they are drivers of many cell-level dynamic processes such as signalling, endocytosis and budding. These ions also strongly modify the physical properties of the lipids that make up a large proportion of cell walls. The precise mechanism behind such effects is poorly understood at the molecular level, where specific ion and hydration effects result in the breakdown of otherwise successful continuum models. Here, we investigate the organisation and dynamics of monovalent alkali ions at the surface of an anionic lipid membrane. We make use of atomic force microscopy (AFM) to detect single adsorbed ions with sub-Ångström and 25 ms spatial and temporal resolution. We show that the ions form non-random condensed patches that evolve slowly over tens of seconds. Individual ions' dynamics are strongly linked to their position within a patch, emphasizing the importance of short-range hydration interactions. Significantly, these ionic domains are shown to induce nanoscale variations in the stiffness of the bilayer, providing a general mechanism for the modulation of the physical properties of biomembranes by the adsorption of monovalent ions.

P-311

Characterization of voltage sensitive dyes with free-standing lipid bilayers

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Voltage sensitive dyes (VSDs) are powerful tools for membrane potential monitoring. The characterization of VSDs is commonly performed either directly with living cells or with vesicles where the membrane potential is established by incorporating ion selective channels. However, both characterization methods lack the possibility to precisely control the voltage sequences, thus are unable to study the kinetics of the dyes. In this work, we demonstrate a new approach for the VSD characterization using free-standing lipid bilayers. First, pores ($\phi = 1 \mu\text{m}$) were fabricated in silicon nitride thin (200 nm) membranes by focused ion beam (FIB). Second, the membrane with pores was mounted in a home-made electrochemical cell where both sides of the membrane is electrically accessible. Third, free-standing lipid bilayers were formed over the pores by the well-known painting method. After the incorporation of VSDs (e.g. di-4-ANNEPS), the fluorescence signal from the bilayer is monitored by fluorescence microscopy while applying different voltage sequences. This platform offers the possibility 1) to apply any voltage sequences, 2) to modify bilayer composition freely, and 3) to acquire two-dimensional mapping of the VSD activities, allowing more detailed studies of VSDs.

Posters**– 7. Nanobiophysics –****P-312****Oxidation/reduction sensing using optical responses of hybrids of DNA and carbon nanotubes**

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Hybrids of DNA and single-walled carbon nanotubes (DNA-SWNTs) were used for quantitative evaluation of antioxidant abilities of Japanese tea and a catechin solution. It is known that near-infrared absorption spectra (NIR-ABS) and near-infrared photoluminescence spectra (NIR-PL) of DNA-SWNT suspension are sensitive on oxidation/reduction of SWNTs. When Japanese tea or catechin was added to DNA-SWNT suspension that was pre-oxidized by 0.03% hydrogen peroxide solution, NIR-ABS and NIR-PL significantly indicated that SWNTs were reduced by the addition. As a result, sensitivity of NIR-PL was almost ten times higher than that of NIR-ABS. Final concentration of catechin was 15 $\mu\text{g/ml}$ in the experiments. Because the Japanese tea also included 15 $\mu\text{g/ml}$ of catechin in final concentration, it is clear that catechin is the major component to determine the antioxidant ability of the Japanese tea. Furthermore, we examined the reduction ability using diluted catechin solution. When the catechin concentration was 0.015 $\mu\text{g/ml}$, NIR-ABS and NIR-PL spectra suggested that DNA-SWNTs were gradually re-oxidized while it was reduced once.

P-314**Investigation of protein clusters in membranes by atomic force microscopy**

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Protein clustering in neuronal systems is a frequently reported phenomenon. It is thought to influence synaptic plasticity and in the presynaptic membrane docking of synaptic vesicles and fusion probability is believed to be altered. Most recent studies use fluorescence techniques in combination with high resolution optical microscopy to study synaptic protein clustering. However, antibodies used in immunofluorescence assay as well as protein modifications might alter the aggregation of these proteins. Therefore, in this study we use a label free approach by atomic force microscopy which exploits the interaction of a specific antibody with proteins like syntaxin present in the membrane of interest, where the antibody is covalently bound to the tip of the atomic force microscopy cantilever. During scanning of the surface of interest force-distance curves are recorded. For this purpose we used artificial membrane systems and plasma membrane sheets derived from adrenal pheochromocytoma (PC12) cells.

P-313**Exploring high affinity of TiO₂ nanotubes to lipid membranes**

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Most studies of interactions between nanoparticles (NP) and biological systems focus on the role of NP-adhering proteins (the so-called protein corona), whereas interaction with lipids is often ignored. The latter is especially relevant for NP that come into contact through lipid-rich environments, such as lungs, or for NP with very high lipid affinity.

As an example of the latter, we show that TiO₂ nanotubes can severely affect the integrity of both cellular and model membranes. Using fluorescence microspectroscopy, we followed accumulation and aggregation of NP on individual giant membrane vesicles. To corroborate the observations, we further employed methods with higher statistical power. Characterization of membrane properties following interaction with NPs using fluorescent polarity-sensitive membrane probes outlined membrane impairment also on the molecular scale, most probably due to increased curvature induced by membrane wrapping around the NP. We additionally confirmed the high affinity of these NPs to lipid membranes by multi-colour super-resolution STED microscopy and single-molecule based fluorescence fluctuation approaches, namely fluorescence cross-correlation spectroscopy (FCCS) and 2D-FIDA.

P-315**Nanozymes and its applications in Biomedicine**

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Nanozyme, a class of nanomaterials with enzyme-like activities, has been widely accepted since the first evidence has been published a decade ago. It has changed the idea that inorganic nanomaterials are chemically inert in biological system, and has directly led to a new concept stating that nanomaterials could be bioactive, which opens up a wide range of applications of nanomaterials for human health and living environment. Here, I would introduce the progress of nanozyme and its latest applications in biomedicine.

Posters

– 7. Nanobiophysics –

P-316

Accelerated molecular dynamics: boosting the probability of rare biological events

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Deoxyribose nucleic acids (DNA) are one of the most important biological molecules in living cells, more specifically, the cell nuclei. Due to their polycationic nature, they readily react with other external molecules through electrostatic interactions. Moreover, since the DNA is a long polymer, which has many internal degrees of freedom, it is also highly susceptible to conformational transitions, in turn having fundamental influences on the functionality of the molecule. Hence a clear understanding of its interaction with other molecules lays the cornerstone for further investigation of other DNA-related biological events, such as mutagenesis and genetic repair mechanisms. However, since these events are extremely rare, with estimated timescales in the regime of milliseconds, which current classical molecular dynamics (cMD) methods cannot achieve, simulations are only possible with special computational techniques which boost the probability of these events whilst preserving the “true” statistical ensemble of the system. In this work, we present a multi-nanosecond simulation of the intercalation process of a biological molecule into DNA, using the method of accelerated molecular dynamics (aMD).

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Nano-mechanical signature of different lung diseasesJ. Zemla¹, T. Stachura², J. Soja², I. Gross-Sondej², K. Sladek², M. Lekka¹

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Abnormalities in cell behaviors such as growth, differentiation and apoptosis are determinants of various diseases. The mechanical properties of cells and tissues can be used as markers for diagnosis of pathological conditions. Here, we use atomic force microscopy (AFM) to evaluate the alterations in mechanical properties of tissue samples originating from patients suffering from asthma, chronic obstructive pulmonary disease and healthy subjects. Collected data reveal bimodal distribution of relative Young's modulus values referring to cellular and fibrous regions present in the tissue. Statistical analysis demonstrated differences between the Young's moduli of healthy and studied groups of pathological tissues.

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Single-molecule solution mass spectrometry by interferometric scattering microscopy

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A variety of methods have been developed over the past decades aimed at the characterisation of the size distribution of proteins and their complexes, including electrophoresis, analytical ultracentrifugation and, most powerfully, mass spectrometry, for studying biomolecular interactions and complex assembly. At the same time, single molecule techniques have enabled access to macromolecular dynamics and structure through fluorescence labeling. Studying protein oligomerisation and interactions of proteins with small molecules at the ultimate, single molecule level without the introduction of external labels, however, has remained challenging.

Here, we show that interferometric scattering microscopy (iSCAT) can not only detect single molecules without any labels, but that it also provides a quantitative measure of their mass in solution with a mass accuracy on the order of a few % of the sequence mass, and that this calibration holds not only for proteins but also for biomolecular complexes containing lipids or carbohydrates. Single molecule solution mass spectrometry by iSCAT opens up completely new avenues to studying the biomolecular interactions.

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Rapid microfluidic dilution for single-molecule spectroscopy of low-affinity biomolecular complexesN. Zijlstra¹, F. Dingfelder¹, B. Wunderlich¹, F. Zosel¹, S. Benke¹, D. Nettels¹, B. Schuler^{1,2}

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To enable the investigation of low-affinity biomolecular complexes with confocal single-molecule spectroscopy, we have developed a microfluidic device that allows a concentrated sample to be diluted by up to five orders of magnitude within milliseconds, at the limit dictated by diffusion. The microfluidic device combines diffusion-limited dilution with multiple, long observation channels offering a wide range of dilution factors for precise tuning of the sample concentrations and observation times ranging from milliseconds to minutes after dilution.

To demonstrate the capabilities of our microfluidic device, we used confocal single-molecule two-color and three-color FRET to study a low-affinity protein complex consisting of the two intrinsically disordered protein domains ACTR and NCBD. We show that this device can be used to transiently populate and study the structural properties of these low-affinity complexes and quantify the dynamics of the dissociation process over a wide range of timescales with single-molecule spectroscopy. The versatility of the device makes it suitable for studying biomolecular complexes with dissociation constants from low nanomolar to more than 10 μ M, covering the vast majority of biomolecular complexes.

Posters**– 8. Forces in and between cells: filaments, membranes and walls –****P-320 (O-52)****Bulk cytoplasmic actomyosin contractions drive streaming in zebrafish eggs**

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At the onset of zebrafish development, the fertilized egg is composed of a mixture of yolk granules (the food supply on which the developing embryo will feed on) and cytoplasm from which all of the embryonic tissues will develop. For development to start, the cytoplasm and yolk granules need to segregate with all the cytoplasm accumulating at one side of the oocyte (animal pole) and the yolk granules on the other (vegetal pole). This segregation process is typically called ‘cytoplasmic streaming or flow’ and the goal of this project is to unravel the physical basis of cytoplasmic streaming within the oocyte.

Previous studies have speculated that reorganization of cortical actomyosin triggers cytoplasmic flows within the oocyte. However, by generating oocytes specifically lacking cortical actomyosin, we were able to show that cytoplasmic streaming also occurs in the absence of the cortical actomyosin network. Instead, we propose that contraction of a previously uncharacterized subcortical actomyosin network can drive this process.

P-322 (O-54)**Stiffening and softening of cytoskeletal networks: rheological insights from minimal systems**

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The cytoskeleton is impressively versatile, manifesting the ability to either stiffen or soften in different stress regimes. A major challenge in cell mechanics has been to identify the mechanistic origin of this behaviour. We address this challenge by preparing minimal in-vitro systems of intermediate filaments and microtubules and probing their mechanical response over different applied stress regimes.

Intermediate filament networks are probed rheologically, increasing the mechanical load with intermittent constant stress. This allows us to probe mechanical response in new high-stress regimes and also to quantitatively measure the network’s remodelling. We reveal a rich diversity of mechanical responses with stiffening at low forces, axial stretching at intermediate forces and a new regime of network softening at high forces.

Through analysis of the network strain over time and by comparing permanent and transient crosslinking agents we reveal that remodelling is driven by crosslinker unbinding and loss of network connectivity. We also present new findings on how the inclusion of microtubules can modulate the onset of these different mechanical regimes, providing new insights into how cells are able to store and dissipate applied mechanical loads.

P-321 (O-53)**Correlative AFM and cryo-EM approach for probing the nuclear lamina mechanics**T. Sapra¹, Z. Qin², M. Buehler², O. Medalia¹¹University of Zurich, Switzerland; ²Massachusetts Institute of Technology, USA

Nuclear lamina forms a shell-like structure in the nucleus. Mutations in the lamin proteins cause disease collectively termed laminopathies. We have developed an approach to study the *in situ* mechanics of nuclear lamins (*Xenopus laevis* oocyte nucleus) at the single filament level, to our knowledge for the first time. This is also the first example of combining AFM and cryo-electron tomography to understand the structural framework of the lamin network, an important step toward combining mechanobiology and visual proteomics. The combination of structural mechanics and network theory analysis provides a credible analysis of the hierarchical organization of the nuclear lamina. The results are in good agreement with molecular dynamics simulations. The study paves the way for further studies of nuclear mechanics in mammalian nuclei.

P-323**Configurations of confined cytoskeletal networks using the monomer ensemble**

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The mechanical properties of a cell depend on the precise nature of networks which make up the cytoskeleton and also on the geometry of the network subject to the confining environment in the cell. There are different types of filaments, these may be bundled, cross-linked, or branched. Therefore it matters not only how these filaments are linked, but also that there are constraints due to the confinement imposed by the cell membrane or cell wall, *i.e.* the filament networks are neither homogeneous nor infinite. We present theoretical calculations that enable the prediction of elastic properties and geometry of such confined networks in several scenarios, but in particular we introduce a simple model for branching of actin with the Arp2/3 complex. The distribution and orientation of filaments within the confining region also vary with position within the [Azari, A. & Müller-Nedebock, K. K. *EPL* **110**, 68004 (2015)]. We show how a generalisation of a monomer ensemble technique [Pasquali, S. & Percus, J. K. *Molecular Physics* **107**, 1303 (2009)] is particularly useful for computational work, leading to density profiles of branching points, and orientations of filaments in dependence of the proximity to cell boundaries.

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– 8. Forces in and between cells: filaments, membranes and walls –

P-324

Mechanical characterization of a trimeric coiled-coil using atomistic simulations

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Coiled-coils (CCs) consist of two or more alpha-helices held together in a bundle. This motif is ubiquitous in many proteins of the cytoskeleton, the extracellular matrix and the viral envelope, and is involved in essential mechanochemical functions such as muscle contraction, cell motility and blood clotting. Most of the extracellular CCs are trimeric whereas their intracellular counterparts are dimeric. This trend suggests a specific, but unknown, mechanical function that is directly related to the CC oligomerization state. Motivated by this observation, we have studied the mechanical response of a synthetic trimeric CC to a tensile force using atomistic simulations and compare its behaviour to a dimeric one with a similar sequence. The force extension-curve of the CC trimer exhibits a nearly constant force plateau which is almost double of the one observed for the dimeric CC. Mutating the hydrophobic and charged amino acids that stabilize the trimeric CC has little impact on the force plateau, suggesting a universal behaviour response of the trimeric CC to tension. We are currently investigating the molecular origin of this universality with network-based analysis methods to characterize the path of force propagation in the CC.

P-326

Mechanic properties of filament network: cytoskeleton, extracellular matrix, ...

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We develop a continuum theory for equilibrium elasticity of a network of crosslinked semiflexible filaments. Analytical expressions are derived for the elastic energy at arbitrary strain, with the corresponding stress-strain relationship. The theory fits well to a wide range of experimental data on simple shear in different filament networks, quantitatively matching the differential shear modulus variation with stress, with only two adjustable parameters (which represent the filament stiffness and the pre-tension in the network, respectively). The theory accurately describes the crossover between the positive and negative Poynting effect (normal stress on imposed shear) on increasing the stiffness of filaments forming the network. We discuss the network stability (the point of marginal rigidity) and the phenomenon of tensegrity, showing that filament pre-tension on crosslinking into the network determines the magnitude of linear modulus. Moreover, the theory has been generalized to discuss about fluidisation of transient filament networks, where the crosslinks, mainly bio-motors, can be dynamically broken and re-crosslinked.

References:

F. Meng, E. M. Terentjev, *Soft Matter* 32, 6749 (2016)

F. Meng, E. M. Terentjev, *Polymers* 9, 52 (2017)

P-325

Red blood cell aggregates in malaria - the role of flow

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Malaria causes about half a million deaths and more than 200 million new patients per year. Most casualties occur due to the adhesion-based sequestration of red blood cells in the microvasculature, which causes blood vessel obstruction and subsequent failure of vital organs. So far, static assays already revealed many biomolecular aspects of the involved adhesion phenomena being linked to the development of the illness. However, they cannot account for the flow-induced shear in the microcirculation. We investigate the influence of shear forces on rosetting and sequestration in vitro employing microfluidic techniques. We developed microfluidic channels to mimic vessels of the microvasculature system to analyze the dynamics of rosettes under physiological flow conditions. Referring to a static reference, we found a large deviation in rosetting frequency under flow and a correlation of rosetting frequency and shear rate. On this basis, we plan to study rosette formation and stability in situ, as well as the interplay of cytoadhesion and rosetting to gain new insights into fundamental mechanisms of the pathophysiology of malaria.

P-327

Dynamics of neutrophil extracellular trap (NET) formation

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Neutrophils are the most abundant type of immune cells in the human blood system and central for innate immunity. Recently, it was found that neutrophils and other cells are able to catch and kill pathogens by expelling a fibril network made from their own DNA (neutrophil extracellular traps, NETs). This process, termed NETosis, is distinct from other forms of cell death such as necrosis and apoptosis and is therefore of central importance for cell biology. During NETosis, a massive rearrangement of the materials inside the cell takes place. So far, the mechanisms that govern this complex process are poorly understood. Here, we show how chromatin, cytoskeleton and membrane structure change the mechanical properties of the cells, which finally leads to rupture and release of NETs. We show that NETosis can be divided into three distinct phases that are mainly governed by entropic swelling of chromatin. DNA passively diffuses out of the disassembled nucleus until it fills the complete cell lumen. Then cells rounds up while they still adhere to the substrate and finally the membrane is ruptured. In summary, these results demonstrate how NETs-release is regulated by material properties of chromatin and membrane.

Posters

– 8. Forces in and between cells: filaments, membranes and walls –

P-328**Combined Optical tweezers & AFM - Investigating cell mechanics & single molecules on multiple scales**

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While optical tweezers (OT) and Atomic Force Microscopes (AFM) have a successful history in biophysical research, a combination that benefits from the advantages of both methods has so far been illusive due to the technical challenges apparent when attempting to merge both techniques.

In some studies, the two methods have been used subsequently on the same type of sample to perform force spectroscopy at different scales. Truly simultaneous optical trapping and AFM measurements, however, have not been available.

Here we present a novel OT-AFM setup that combines high precision optical manipulation, camera-based force detection and the full spectrum of AFM methods on the same sample at the same time. This opens up new approaches to complex experimental designs that are not accessible by AFM or OT alone. It was used in immune-biology experiments to characterize the influence of dendritic cell + T-cell interaction on cellular adhesion. In addition to results from these measurements, we will introduce further application examples from the field of single-molecule interactions, cell mechanics and medicine.

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Posters

– 9. Systems biology –

P-329 (O-57)

Applications of stochastic lumping analysis to fluctuations in systems and structure biology

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Conventional studies in systems and structure biology rely largely on the construction of kinetic schemes. Since the selection of these networks depends on the level of coarse-graining and thus is not unique, a concern is raised whether and under which conditions hierarchical schemes can reveal the same experimentally measured fluctuating behaviors and unique fluctuation related biological properties. To clarify these questions, we introduce stochasticity into the traditional lumping analysis, generalize it from rate equations to chemical master equations and stochastic differential equations, and extract the fluctuation relations between kinetically and thermodynamically equivalent networks under intrinsic and extrinsic noises. The results provide a theoretical basis for the legitimate use of low-dimensional models in the studies of biological fluctuations and, more generally, for exploring stochastic features in different levels of contracted networks in chemical and biological kinetic systems. [J. Chem. Phys. 142, 184103 (2015)]

P-330 (O-58)

Escherichia coli's strategies for maintaining proton motive force when exposed to photodamage

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Fluorescent microscopy is a powerful tool for visualization and dynamic observation of cell's characteristics (size, shape, internal pH *etc*). However, repeated exposure of fluorescently labeled cells to illumination from lasers or high-intensity lamps and LEDs can lead to photodamage and changes in cell physiology. The damage is attributed to reactive oxygen species (ROS) formed during cell exposure to light. It has been shown that ROS formation inhibits cell growth, damages the membrane and causes DNA breaks. Despite these findings, the immediate response of live cells to photodamage has not been identified. Here we combine epi-fluorescence and back focal plane interferometry to simultaneously monitor, in individual cells, two components of *Escherichia coli*'s energetics – proton motive force (PMF) and internal pH. We demonstrate that under photodamage *E. coli* cells lose PMF in a fashion analogous to a capacitor discharging if kept in nutrient deprived buffer. Interestingly, in nutrient rich media, *E. coli* maintains, or even increases, the PMF in response to photodamage at the expense of severe loss of internal pH. We present a mathematical model that offers a possible explanation for our observations and discuss the model predictions for the overall cell energy maintenance.

P-331 (O-59)

Chemically-driven kinetics of phase separated membrane-free organelles

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Liquid-liquid phase separation of proteins from cytoplasm allows the cell to spatially organise its interior by assembling membrane-free, liquid organelles. In equilibrium fluids, a collection of droplets is thermodynamically unstable and coarsen via Ostwald ripening and coalescence. In the non-equilibrium cellular environment, it is unclear how membrane-free organelles are stabilized. We study theoretically the effect of non-equilibrium chemical reactions on a phase separated ternary mixture, composed of a two-state protein and cytoplasm. One state is prone to phase separate, the other state is soluble, and first order chemical reactions convert one state into the other, and vice versa. By controlling the reaction rates, one can bring the system into a stable, monodisperse droplet regime, where Ostwald ripening is arrested, or into an unstable regime with active Ostwald ripening. We predict a relationship between the reaction rates, and protein concentration gradients inside and outside droplets, and the droplet sizes. Finally, we apply our results to stress granules, a class of membrane-free organelles that form during various environmental stresses.

P-332

Fluorescent probes to evaluate membrane properties of multidrug-resistant isolates of *E. coli*

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The ability of most compounds (nutrients and antibiotics) and ions to cross the bacterial cytoplasmic membrane by diffusion and active transport is highly dependent on cytoplasmic membrane fluidity, which can be determined using fluorescent probes to estimate membrane anisotropy and polarization values. In this study we aimed to evaluate those biophysical properties in two multidrug-resistant isolates of *Escherichia (E.) coli* in comparison to a susceptible *E. coli*. The studies were performed throughout time (after 24, 48 and 72 h of incubation) and in the absence and presence of antibiotics. Two antibiotics were used, cefotaxime and ciprofloxacin, which were added to the inoculum at concentrations equal to $\frac{1}{2} \times \text{MIC}$. Accordingly, for each experimental condition, the membrane fluidity from fluorescence anisotropy of DPH-labelled cells as well as the Laurdan generalized polarization, at 37°C, were determined.

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– 9. Systems biology –

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Protective assessment of Phikud Navakot against hypoxia/reoxygenation-damaged H9c2 cardiomyoblasts

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Phikud Navakot, a combination of nine medicinal plants, is a major component of “Yahom Navakot” using as traditional Thai medicine for treatment of dizziness and fainting. The present study was aimed to investigate the cardioprotective effect of the ethanolic extract of Phikud Navakot (PN) on hypoxia/reoxygenation (H/R)-damaged H9c2 cells. Cardioprotective ability of PN was measured after 6-h exposure to hypoxic buffer in ischemic box followed by 24-h reoxygenation in complete medium in CO₂ incubator. The results showed that PN at a concentration of 0.1 mg/mL was able to protect cell injury observed by phase contrast microscopy when compared with the H/R control. PN at the same concentration also inhibited DNA condensation determined by Hoechst 33342. However, immunoblotting showed that PN decreased the phosphorylation of ERK1/2 when compared to the H/R control. The protective ability of PN against HR injury is further investigated. In conclusion, the present results revealed that PN (0.1 mg/mL) was able to protect H9c2 cardiomyoblasts injury induced by H/R.

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Selective response to specific ligands in T cell ligand discriminationM. K. Kajita¹, K. Aihara^{1,2}, K. J. Tetsuya¹

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Cells make decisions based on the environmental information, which is coded by the presence or concentration of a target ligand for the cells. To sense the environmental information, cells recognize the target ligand using cell surface receptors. However, in the environment, there are similar non-target ligands. Although the target ligands have high specificity to the receptors, the non-target ligands also have some specificity to the receptors, which enables the non-target to bind to the receptors. To generate a selective response to the target ligand, cells must have a mechanism to discriminate the target from the non-target ligands. As a mechanism of the ligand discrimination, specificity amplification by multi-step discriminatory reactions has been proposed. Although the mechanism or the extended mechanisms can explain several aspects of the ligand discrimination, other mechanisms without relying on the multistep reaction have not been fully understood. In this research, we propose a ligand discrimination mechanism, which can amplify specificity without based on the multistep reaction. We show that the mechanism can reproduce the qualitative characteristics observed in recent bio-imaging. We also discuss the connection between concentration sensing and ligand discrimination.

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Does the DNA damage response depend on growth in bacteria?S. Jaramillo-Rivera¹, M. El Karoui^{1,2}¹Institute of Cell Biology, University of Edinburgh, UK;²SynthSys, University of Edinburgh, UK

The toxic effect of many antibiotics includes damaging cellular DNA. Bacteria responds to DNA damage by activating transcription of the SOS regulon, which can lead to increased mutagenesis and antibiotic tolerance. Many SOS inducing factors, such as the frequency of replication-dependent DNA damage and the capacity of cells to induce gene expression, are depend upon cell physiology, but the extent of these dependencies is still largely unknown. To address this question, we explored the consequences of growing bacteria in different nutrient media on the response to DNA damage. By quantifying the distribution of SOS induction using fluorescent microscopy and microfluidics, we have found that the intensity and the variability of the SOS response is inversely correlated to growth rate. Furthermore, under slow growing conditions, we observed a second population of highly induced cells, which is absent in fast growing conditions. We explored the potential mechanisms involved in growth dependent bifurcation of the SOS response and how they may change antibiotics tolerance. Our findings indicate that bacteria responds more strongly to DNA damage at slower growth rates, and highlights the relevance of both cell physiology and variability in the context of stress responses.

P-336

Enzyme activity at lipid membranes – correlation of activity and membrane stateA. Kamenac^{1,4}, A. Wixforth^{1,4}, T. Obser², S. Reinhard², M. F. Schneider³, C. Westerhausen^{1,4}

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Enzymes are crucial to form and maintain life by catalyzing slow and unspontaneous chemical reactions. This study regards the activity of the water soluble enzyme ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) bound to large unilamellar phosphatidylcholine vesicles mimicking physiological and cellular conditions in a first order approach. The enzymes bound to the lipid membranes exhibit a temperature dependence of their activity in strong correlation with the phase state of the membrane. As a result, the temperature-dependence of the activity is not purely Arrhenius, but shows an additional peak at the phase transition temperature of the according lipid membrane. By exchanging the lipid mixture from 14:0PC to 15:0PC this activity peak again is present, but shifted towards higher temperatures and again matches the phase transition temperature. This is in accordance with the theory of Konrad Kaufmann that predicts a correlation of increased system fluctuations, as present at the phase transition, and the activity of an enzyme.

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– 9. Systems biology –

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Predicting bacterial growth in response to antibiotic combinations using growth lawsB. Kavčič¹, G. Tkačik¹, T. Bollenbach^{1,2}¹Institute of Science and Technology Austria, Austria;²Institute for Theoretical Physics, University of Cologne, Germany

Rapid growth of bacteria requires a well-orchestrated translation machinery which is modulated internally by translation factors and perturbed by certain antibiotics (translation inhibitors). Coupling of the growth rate and ribosome synthesis results in ribosomal growth laws - relations connecting ribosome concentration to the growth rate. We applied these to build a model of bacterial growth in the simultaneous presence of translation factors and various antibiotics. Joint effects of antibiotic combinations range from synergistic to antagonistic and are difficult to predict, as the mechanisms of these interactions remain unknown. We hypothesize that the interactions between antibiotics arise from their kinetic properties together with the interplay of different stages in which ribosomes are halted. To test how halting of the ribosomes affects the efficacy of antibiotics, we constructed bacterial strains in which we control the abundance of different translation factors. Measurements of interactions between antibiotics and translation factors verified our mathematical model and showed how antibiotic efficacy depends on the targeted process in the translation cycle. These results provide new insights into the mechanisms of translation inhibitors and their combinations.

P-339

The F-fraction: The variation in allocation of resources to flagella production in *Escherichia coli*

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Like many bacteria, *Escherichia coli* is propelled forward by about half a dozen flagella bundled together. Each flagellum is a long and complex structure (>20 000 proteins) consisting of the bacterial flagellar motor, a hook and filament proteins. In many situations mobility is advantageous to the bacterium, allowing it to 'escape' a localised area of stress or find areas of higher nutrients. However, when the bacterium is in a rich nutrient or low stress region, motility is less useful. Given the number of proteins in each flagellum, its production requires significant energy consumption, thus, in these cases, the flagellum acts as a 'burden' upon the cell.

Here we construct two *E. coli* strains, identical except for their ability to produce filaments (consisting of ~20 000 copies of the same protein). We identify differences in number of flagella per bacterium across the population in a range of typical growth conditions and from population variance and dependence of flagella numbers on growth conditions we hope to understand how *E. coli* decide to invest in flagella production (here we call it the F-fraction of the total proteome). Such understanding would offer valuable insight into allocation of resources across the proteome and feedback mechanisms that govern these.

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Synchronous oscillatory network and cholinergic system in the slug olfactory center

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Synchronous oscillatory activity in a laminar structure is common in the central nervous system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC), olfactory center, and its frequency changes are suggested to encode the olfactory information and memory. We recently found that *in vitro* oscillatory neuronal network was formed from dispersed cell culture of PC neurons. In the present study, we thus examined what role cholinergic system plays in synchronized oscillatory network of cultured PC neurons. First, increases in neurite arborization and neurite connection were observed after a week in culture. Second, in calcium imaging for the PC neuron network, nicotine or acetylcholine esterase inhibitor increased the number of spontaneous calcium transients in PC neurons and induced synchronous oscillatory activity in the network. Cholinergic systems involved in a higher cognitive function of the brain of various species could be function as a major transmitter in cultured PC neuron network, and furthermore, may play an essential role for synchronous oscillation in the olfactory neuron network.

P-340

Muscarinic receptors are responsible for the cholinergic modulation of projection neuronsW. Meng^{1,2}, S. Wang^{1,2}, L. Yao^{1,2}, N. Zhang¹, D. Li¹¹South China Normal University, China; ²Jiangxi Science and Technology Normal University, China

Songbirds are a useful model for the study of learned vocal behavior in vertebrates. The robust nucleus of the arcopallium (RA) is a premotor nucleus in the vocal motor pathway. It receives excitatory synaptic inputs from the anterior forebrain pathway. RA also receives cholinergic inputs from the ventral paleostriatum of the basal forebrain. In the present study, we investigated the electrophysiological properties of two acetylcholine receptors on the RA PNs of adult male zebra finches using *in vitro* whole-cell current clamp. Our results demonstrate that activation of muscarinic acetylcholine receptors (mAChRs) simulate the effects of carbachol. Both carbachol and the mAChR agonist muscarine produced a decrease in the excitability of RA PNs and a hyperpolarization of the membrane potential. The mAChR antagonist atropine blocked the effects of carbachol. Activation of nicotinic acetylcholine receptors (nAChRs) with nAChR agonist nicotine or DMPP had no effect on the excitability of RA PNs, and the nAChR antagonist mecamylamine failed to inhibit the effects of carbachol. These results suggest that mAChRs, but not nAChRs, primarily modulate the effects of carbachol on the activity of RA PNs. Collectively, these findings contribute to our understanding of the mechanism of cholinergic modulation in the vocal nuclei of songbirds.

Posters

– 9. Systems biology –

P-341

Cardioprotective potential of *Ligusticum sinense* on hypoxia/reoxygenation injury in H9c2 cellsP. Nusuetrong¹, O. Gerdprasert²¹Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, 10110, Thailand; ²Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, 10110, Thailand

Ligusticum sinense has been used for treatment of inflammatory and coronary heart diseases, headaches, swelling and pain. The aim of the present study is to investigate the protective effect of aqueous extract of *L. sinense* (LS) on H9c2 cardiomyoblasts against hypoxia/reoxygenation (H/R) injury. The cytotoxic and protective effects of LS on H9c2 cells were measured by MTT reduction assay. Incubation of LS at the concentrations between 0.01–1 mg/mL for 24 h showed no toxic effect on H9c2 cells. Exposure of LS (0.1 mg/ml) during 6-h hypoxia in hypoxic buffer followed by 24-h reperfusion in complete medium was able to increase cell survival when compared with normoxia control. In addition, LS at the same concentration was able to inhibit DNA condensation measured by Hoechst33342 staining when compared with H/R-exposed cells. The results suggested that the aqueous extract of *L. sinense* may play a partial role in protection of H9c2 cells against H/R injury.

P-343

General calibration of optical density measurements for microbial growth

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Optical density (OD) measurements of microbial growth are one of the most common techniques used in microbiology, with wide-ranging applications such as studies of antibiotic efficacy, investigations of growth under different nutritional or stress environments and characterization of different mutant strains, including those harbouring synthetic circuits. These OD measurements are performed under the assumption that the measured OD value is proportional to the cell number, i.e. the concentration of the sample. However, in many applications, an additional factor, such as cell size, varies during the growth of the culture. Here we examine how three such factors, cell size, refractive index and sample heterogeneity, alter the measured OD independent of growth in the sample. We show that these effects invalidate the assumption that OD and cell concentration are proportional to one another and that this can lead to errors in determining cell concentrations. Additionally we show how a series of simple calibration methods can largely mitigate these factors allowing for more accurate reporting of cell numbers in bacterial cultures.

P-342

Investigation of relations between GSTT1 polymorphism and lower extremity varixD. Saribal Kanber¹, N. Bahtiyar¹, M. A. Kilic², E. M. Kanber³, A. K. Kirektepe Aydin⁴, S. Toplan¹, M. C. Akyolcu¹¹Istanbul Uni. Cerrahpasa Med. Fac., Dep. of Biophysics, Turkey; ²Adnan Menderes Uni. Med. Fac., Dep. of Biophysics, Turkey; ³Gaziantep Ersin Aslan Hospital, Dep. of Cardiovascular Surgery, Turkey; ⁴Istanbul Technical Uni, Molecular Biology and Genetical Research Center, Turkey

Varicose veins of the lower extremity (VVLE) are a frequently encountered vascular disorder in the general population, but etiology of VVLE has not yet been fully explained. This study is planned to answer at least part of the question of whether the vein wall loses function and/or the deficiencies in its function are caused by deficiencies in genetic defense systems. For this purpose, the GSTT1 gene polymorphism encoding the enzyme GSTT1 responsible for the metabolism of cytotoxic agents, which plays a physiological role in initiating the detoxification of potential alkylating agents, has been investigated. Patient group blood samples were taken during the surgery of who had primary varixes of the saphen vein. Control group blood samples were taken during the graft surgery of the patients who had coronary arterial graft operation. DNA were used for polymorphism analysis in GSTT1 gene (qRT-PCR). The appearance of this band on electrophoresis shows that the genotype is intact, that is the T wild genotype. There was no statistically significant relationship between the GSTT1 null genotype in control group with patient group.

P-344

Effect of early ischemia on ventricular action potentialR. Treinys, R. Mačianskiene, M. Almanaityte, J. Jurevičius
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Metabolic stress evoked by myocardial ischemia leads to impairment of heart function. We investigated the effect of induced early ischemia and the changes of the action potential parameters in the isolated rabbit heart. The suppression of L-type calcium current during ischemia is usually reported effect but our experiments revealed a dual response of calcium current to the uncoupling of oxidative phosphorylation. When ventricular cells were exposed to uncoupling agents, it induced a biphasic effect on L-type calcium current. Uncoupling evoked a rapid initial stimulation that was followed by a strong inhibition of L-type calcium current. We tested how this phenomenon is reflected in the action potential of isolated rabbit heart. Results showed that in the early phase of ischemia the action potential duration (APD) was briefly increased. At 20% repolarization ventricular APD increased up to 10% after 0.5 min of ischemia. This increase of APD was followed by a typical gradual decrease of APD when ischemia progresses. We attribute this dual change of APD to the biphasic changes of L-type calcium current and calcium transient increase in the early stage of ischemia in the heart. This research is funded by a grant (No. MIP-058/2015) from the Research Council of Lithuania.

Posters**– 9. Systems biology –****P-345****Effects of selenium supplementation on cytokines in experimental hyperthyroidism**

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Background: Even though selenium is an essential trace element, higher doses might have toxic effects. Selenium has regulatory effect on endocrine functions, role in antioxidant defense and also performs function in inflammatory incidents. So it may be evaluated that selenium has important efficiency in organism. In our study it has been investigated that if selenium supplementation has any effect on inflammatory cytokines as TNF-alpha and IL-6 in experimental hyperthyroidism.

Method: Hyperthyroidism was induced in wister albino rats by oral intake of L- thyroxin. Animals were exposed to different doses of selenium for 30 days. TNF-alpha and IL-6 levels were determined by ELISA. The hyperthyroid group values were compared with control and hyperthyroid groups with selenium supplementation.

Findings: TNF- alpha and IL-6 levels were found higher in hyperthyroid group comparing to control group. A significant decrease of TNF-alpha and IL-6 levels in hyperthyroid groups with selenium supplementation were measured comparing to hyperthyroid group. It may be said that selenium supplementation in hyperthyroidism may have regulatory effect in inflammatory system and it possibly can be used in treatments for hyperthyroidism.

Posters

– 10. Quantum biology –

P-346 (O-62)

Quantum vibrational excitations and protein folding *in vivo*

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One main hypothesis is that when triggers like water molecules or ions or other ligands, or chemical reactions, act on a protein, the energy input to the protein is in the form of local quantum vibrational excited states (the VES hypothesis). Simulations show that, **as quantum states**, these VES can hop **without dissipation**, from the active site, where they are generated, to other sites in the proteins. This presentation is concerned with the application of the VES hypothesis to protein folding. According to mainstream theories, the native state of proteins is that which minimizes its (free) energy. Instead, the view here is that the native state is just one of the many stable kinetic traps into which the same protein may fall. In this perspective, protein folding is a kinetic process and folding reproducibility requires not only that the pathway followed by the protein from the initial conformation is always the same, but also that the initial conformation is always the same. My hypotheses are that the nascent chain of **all** proteins is helical and that the first step in protein folding is the bending of that initial helix at specific sites. It is shown that the application of the hypotheses described above can lead to a complete picture of the folding of a small all-alpha protein.

P-348

2-D Electronic Spectroscopy on the light-dependent enzyme protochlorophyllide oxidoreductaseE. Romero², D. A. Farmer¹, C. N. Hunter¹, R. van Grondelle²¹University of Sheffield, UK; ²Vrije Universiteit, Netherlands

In photosynthesis, quantum coherence in light harvesting complexes could form the basis for the highly efficient propagation of excited states within the photosynthetic membranes of bacteria and plants. Recent studies on Photosystem II reaction centres extends the possible relevance of electronic coherence to the photochemical steps of photosynthesis; it was shown that coherent states are sufficiently long-lived, at room temperature, to persist during the initial steps of electron transfer. This extension of quantum coherence, from the energy transfer to the energy trapping steps of photosynthesis, made it timely to investigate if quantum coherence also plays a role in the formation of product states in enzyme catalysis. Given the short timescales involved it was necessary to study an enzyme where the catalytic cycle can be triggered by femtosecond light pulses, so protochlorophyllide oxidoreductase (POR) was selected as an ideal model system. 2-dimensional electronic spectroscopy (2DES) was used to investigate the presence of quantum coherence in the intermediates of the light-dependent reduction of protochlorophyllide (Pchl_{id}) to chlorophyllide (Chl_{id}) catalysed by POR.

P-347

Intracellular local temperature as a novel variable in cell biologyK. Okabe^{1,2}, M. Takinoue^{2,3}, K. V. Tabata^{1,2}, T. Funatsu¹¹University of Tokyo, Japan; ²PRESTO, JST, Japan; ³Tokyo Institute of Technology, Japan

Temperature, a key regulator of biochemical reactions, defines the thermodynamic aspects of the intracellular environment and controls cell functions. We previously demonstrated monitoring and imaging of intracellular temperature based on a fluorescent polymeric thermometer and quantitative fluorescence imaging techniques, showing intracellular temperature variation in space and time associated with cellular functions. These findings, especially in-homogenous temperature distribution in steady-state cells, are distinct from pure solutions, attracting great attention in physical and biological perspective. However, the mechanism and the significance of temperature change in cells are poorly understood. Here, by utilizing local heating techniques for a single living cells, we directly observed the dynamics of temperature change. The quantitative transient heating allowed examinations into the mechanism of temperature variation inside of cells, which shows a unique property of intracellular heat dynamics. These result suggested a unique chemical environment of intracellular space relating the thermodynamics, rendering the temperature variation in cells markedly large, contributes to intracellular thermogenesis-mediated cell functions.

Posters

– 11. Computational biophysics –

P-349 (O-26)

Beyond sequence: implications of DNA structure and dynamics in genome function

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DNA looping and folding are essential features for the function of genomes, highlighting the importance of studying the physical properties of DNA and its interacting molecules.

I have explored the length-dependence of DNA elasticity to understand the origin of the contradictory experimental measurements obtained at the micro and meso-scale. While classical single-molecule force-extension techniques described DNA as a rigid polymer, novel experimental methods have suggested DNA is more flexible at smaller scales. I found that DNA elasticity varies on the molecule's length in surprising ways, thus unravelling the experimental contradiction [1].

Moreover, I have been interested to describe how DNA deformations can be transmitted through the DNA fibre itself or across DNA loops in a 1D or 3D mechanism of communication, respectively. In the former, we detected that the probability for a DNA side to melt not only depends of local sequence but also of the whole topological domain [2]. In the latter, I found the enhanced capacity of supercoiled DNA plectonemes to promote protein-DNA interactions in relation to relaxed loops [3].

1. Noy et al, *Phys Rev Lett*, (2012), 109, 228101.
2. Sutthibutpong et al. *Nuc Acids Res*, (2016). 44, 9121.
3. Noy et al. *Biophys J*, (2017), 112, 523.

P-351 (O-68)

Structural dynamics of monomeric alpha-synuclein on the ps-μs time scale derived from MD simulationsR. Klement¹, T. M. Graen¹, A. Grupi², E. Haas², H. Grubmüller¹

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α-synuclein[aS] is an intrinsically disordered protein[IDP], which can form pathogenic β-sheet aggregates. A number of dynamically reordering regions exist, in which secondary structure elements form and melt on ns-μs timescale. Here we used molecular simulations to quantify the timescales at which the aS monomer reorders. Both structure and dynamics of IDPs are very sensitive to force field inaccuracies. We therefore performed extensive test simulations using four force field /water model combinations and compared the results to NMR, SAXS and FRET data. We find that secondary structure forms and dissolves on time scales of a few μs, very similar to those of tertiary structure rearrangements. Remarkably, both largest β-sheet formation rates and propensities were seen for the NAC region, involved in fibril formation. Since the on-rate for β-sheet aggregation is much slower, a conformational selection mechanism seems plausible for aggregation. We also investigated the spontaneous α-helix formation in the structurally variable region between residues 1-100. The on-rates for α-helix formation are very low or not observed at all. This suggests an induced fit or much slower conformational selection mechanism for α-helix formation upon membrane binding compared to β-sheet formation.

P-350 (O-67)

Codon recognition on the ribosome - free energy and QM/MM calculations

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Classical free energy calculations have been combined with QM and QM/MM calculations of the affinity of codon-anticodon interactions in the ribosomal decoding site, where several crystal structures [1,2] indicate that an anticodon base in the wobble position, assumes the normally high-energy enol form when it is involved in a non-cognate base pair. Our previous classical free energy calculations[3], which do not account for the cost of the keto->enol conversion, showed that the extra hydrogen bond provided by the enol form would indeed stabilize the non-cognate base pairs. We have now extended these results with QM/MM calculations in the ribosomal context.

[1] A. Weizlbaumer, F.V. Murphy, A. Dziergowska, A. Malkiewicz, F.A.P Vendeix, P.F. Agis, V.

Ramakrishnan, *Nat Struct Mol Biol* 14 498 (2007).

[2] N. Demeshkina, L. Jenner, E. Westhof, M. Yusupov, G. Yusupova, *Nature* 484 256(2012)

[3] O. Allnér, L. Nilsson, L. RNA 17 2177 (2011)

P-352 (O-69)

Getting the ion-protein interactions right in Molecular Dynamics simulations

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Ion-biomolecules interactions are ubiquitous and play a central role in a number of fundamental biological processes, such as calcium signaling and insulin storage in the ion-rich secretory granules. However, assessing the ion-biomolecule interactions is difficult because of the complexity of the systems involved: quantum descriptions are too expensive to study such large systems, and solvation properties of divalent cations are poorly reproduced by standard force fields, because of polarization and charge transfer effects.

Our goal is to improve the description of cations in simulations and apply it to biologically relevant problems. We obtain reference data for the interaction of non transition metal divalent cations (Ca²⁺, Mg²⁺, Zn²⁺) with typical protein groups on small model systems, which we study combining *ab initio* Molecular Dynamics simulations and neutron-scattering experiments. This leads to the development of a scaled charge description of the ions, which takes into account electronic polarization in a mean field way [1]. The obtained force field is applied to the interaction of ions with the insulin molecule in different multimeric states.

[1] Kohagen M., Mason P.E., Jungwirth P., *J. Phys. Chem. B*, 2014, 118, 7902-7909

Posters

– 11. Computational biophysics –

P-353

Molecular dynamics simulation of engineered β -strand peptide interaction with AqpZ membrane proteinM. Aminpour^{1,2}, H. Hoi^{1,2}, C. Montemagno^{1,3}¹Dept. of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, T6G 2R3, Canada; ²Ingenuity Lab, Edmonton, Alberta, T6G 2R3, Canada; ³Ingenuity Lab, Edmonton, Alberta, T6G 2R3 and National Institute of Nanotechnology, Edmonton, Alberta T6G 2R3, Canada

β -strand peptides can be used to stabilize integral membrane proteins (IMPs); sequestering the hydrophobic surfaces by forming ordered, stabilizing β -barrel-like structures [1]. We are able to design and build hybrid systems crosslinking functionalized β -strand:protein complex to a variety of matrix materials, such as polymers and glass experimentally. The resulted protein-incorporated biomimetic membranes can be used for industrial purposes. Understanding the mechanism underlying β -strand formation and β -strand:protein complexes at the molecular level is essential to control the formation and orientation of the embedded complex in the hybrid device. In this study, AqpZ, the water channel from *E. coli*, is used as a model protein. We employed a systematic computational approach including molecular dynamics simulations to study the β -strand formation and interaction with the protein, paving the way to rationally design β -strand peptide variants with improved stoichiometric and oriented crosslinking ability on AQP's.

[1] Nat Meth, 2013. 10(8): p. 759-761.

P-355

Study of a fluid-gel transition process in a lipid bilayer under the influence of an external electric fieldA. Bartocci¹, L. Monticelli², G. Rossi¹¹Department of Physics, University of Genoa, via Dodecaneso 33, 16146 Genoa, Italy; ²Molecular Microbiology and Structural Biochemistry (MMSB), CNRS UMR 5086, 7 Passage du Vercors, 69007 Lyon, France

The study of nanoparticles (NPs), their size and their shape has increased importance in many biomedical applications, because of their abilities to pass the phospholipid layer. It is important, hence, to understand how NPs interact with the lipid membrane, and how their surface functionalization drives this interaction. More in details, charged NPs interact with the heads of lipids, perturbing the bilayer, and leading to NP adsorption or bilayer rupture. Wang et al., recently, have experimentally noticed that the adsorption of negatively charged NP onto the surface of phospholipid layer has restructured the local bilayer phase, promoting a shift from the fluid to the gel one [1]. Through Molecular Dynamics (MD) calculations, we decided to analyze the problem, in order to better focus on the phase shift and on how it occurs under such conditions, by simulating the interaction of the lipid bilayer with an external electric field, amenable to that generated by a negatively charged NP.

[1] Wang, Bo and Zhang, Liangfang and Bae, Sung Chul and Granick, Steve; *PNAS*, **105**, 18171-18175

P-354

The order-disorder transition in proteins is a jamming transition

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The order-disorder transitions in grains and glasses, two widely different materials, occurring upon increasing temperature and external load, respectively, have common features. They lead to a universal jamming phase diagram conjecture, but unified theories are lacking, mainly because of the disparate nature of the particle interactions. In my talk I will discuss how the order-disorder transition *in proteins* exhibits signatures common to both glassiness and jamming by using molecular dynamics simulations. Ordered protein regions develop a peak in the interatomic force distributions that is universal with those of jammed matter. Dynamical signatures are found as a dramatic slowdown of stress relaxation upon folding, and a picture of the role of internal interactions emerges. Secondly, in my talk I will also discuss how order parameters (of the type that are typically measured in NMR relaxation) which measure motional disorder at the bond level, are affected by order-disorder transitions and I will link their motional change to a sea-to-lakes transition of the underlying free energy surface. Results have implications for designing stable biopolymers.

(1) P. P. Jose and I. Andricioaei, *Nature Communications* **3**, 1161 (2012)(2) A.T. Frank, Q. Zhang, H.M. Al-Hashimi, I. Andricioaei, *Biophys. J.* **108**, 2876 (2015)

P-356

A new protocol to improve the predictive power of molecular dockingA. Basciu¹, F. Pietrucci², A. M. Bonvin³, A. V. Vargiu¹¹Dipartimento di Fisica, Università di Cagliari, Italy;²Université Pierre et Marie Curie, Paris (UPMC), France;³Bijvoet Center for Biomolecular Research, Utrecht University, Netherlands

Quantitative understanding of molecular recognition is key for basic research and computer-aided drug design projects. Docking mimics ligand-receptor association *in silico*, providing an atomic-level model structure of their complex. Unfortunately most docking algorithms underestimate receptor flexibility, reducing the rate of success when binding induces large structural changes of partners. Ensemble-docking, where a set of receptor structures (e.g. from MD simulations) is considered, was implemented to overcome this limitation. Clearly, ensemble structures should include conformations prone to host ligands (holo form), which is usually not the case when apo and holo forms are separated by high free energy barriers. To improve generation of holo-like receptor conformations starting only from its apo form, we implemented a computational protocol based on enhanced-sampling MD simulations. We validated our method on proteins whose apo and holo structures were available and previous efforts to generate holo-like structures and native-like docking poses failed. Receptor structures obtained with our method were comparable to those extracted from MD trajectories of the complex. Furthermore, the docking poses generated by using these structures were native-like and top-ranked in score.

Posters

– 11. Computational biophysics –

P-357

Prediction of complex structure and affinity of CDK2 and its inhibitor using McMD and TI simulationsG.-J. Bekker¹, N. Kamiya², M. Araki³, I. Fukuda¹, Y. Okuno³, H. Nakamura¹¹Osaka-U, Japan; ²U-Hyogo, Japan; ³Kyoto-U, Japan

We have performed multicanonical molecular dynamics (McMD) and thermodynamic integration (TI) simulations to accurately predict the bound complex and their affinity for cyclin-dependent kinase 2 (CDK2) and one of its inhibitors, CS3. CDK2 is involved in cell cycle regulation. Malfunctions in CDK2 are thought to cause tumorigenesis, and thus it is a potential drug target. Here, we have performed a McMD simulation for docking the inhibitor CS3 to CDK2 starting from an unbound structure. From the free energy landscape at 300 K, stable binding configurations can be identified. However, due to a limited amount of stable structures in the unbound region, the affinity is difficult to be determined from the McMD simulations. TI however can efficiently be used to calculate the affinity by measuring and integrating the averages forces on the ligand along a pathway. A potential binding/unbinding pathway and structures along the path were given from the McMD trajectory. Subsequently, the binding free energy was readily computed by TI along the pathway starting from structures obtained from the structure ensemble by McMD along the binding/unbinding pathway. Using this combination, the correct binding configuration was reproduced, and their affinity coincided well with the experimental value.

P-359

Interaction of hydrophobic polymers with model lipid bilayersD. Bochicchio¹, E. Panizon¹, L. Monticelli², G. Rossi¹¹Physics Department of the University of Genoa, Italy;²CNRS, UMR 5086, Lyon, France

Plastic nanoparticles (NPs) are generated in the environment by degradation of everyday-use plastics. The assessment of the risks associated to the interaction of micro and nanoplastics with living organisms, especially in the marine environment, is a major challenge. The study of the interactions between nanoplastics and living cells, though, is still lacking a molecular perspective. Here we use atomistic and coarse-grained molecular simulations to investigate the interaction of polyethylene (PE), polypropylene (PP) and polystyrene (PS) with POPC membranes. PE, PP and PS NPs with size up to 7 nm enter easily the hydrophobic core of lipid membranes. PP and PS disperse in the bilayer [1], while PE shows a tendency to aggregate [2]. We also examined the interaction of the polymers with heterogeneous membranes characterized by liquid-ordered/liquid-disordered phase separation. The behavior of the three polymers is markedly different: PP disfavors lipid phase separation, PS stabilizes it, and PE modifies the topology of the phase boundaries [1,2]. Hydrophobic polymers thus have major effects on the properties of lipid membranes, calling for further investigations on model systems and cell membranes.

[1] Rossi et al., JPCLett 5 (2014) 241

[2] Bochicchio et al, submitted

P-358

Investigating the beginnings of material-driven fibronectin fibrillogenesis with MD simulationsM. Bieniek^{1,2}, W. Taylor¹, C. D. Lorenz²¹Computational Cell and Molecular Biology Laboratory, The Francis Crick Institute, UK; ²Department of Physics, King's College London, UK

The glycoprotein fibronectin (FN) is an important building block of extracellular matrix (ECM). FN connects the intracellular skeleton of a cell to the ECM using the integrin receptors. When binding to the integrins, the FN undergoes a crucial conformational change from a compact form to an extended one, which is necessary to start FN fibrillogenesis leading to the formation of an interconnected network.

In 2009 Prof. Manuel Salmeron-Sanchez developed material-driven fibrillogenesis to create a FN network was proposed with the use of polymers and the cells are not present. The formation of the FN network is achieved by coating the poly(ethyl acrylate) substrate with FN]. However, when a polymer that differs by a single carbon, poly(methyl acrylate), is used instead a FN network does not form.

In this work, we present a series of molecular dynamics simulations that we use to investigate the adsorption of multi-domain portions of the fibronectin protein which are believed to play a key role in this fibrillogenesis process to a variety of functionalised interfaces. In doing so, we will present the peptide-material interactions that are key to allowing or blocking the fibrillogenesis to occur, which will also be compared to experimental results.

P-360

Multi-scale simulations of focal adhesion kinase at PIP containing membranes

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Phosphatidylinositolphosphates (PIPs) are responsible for recruiting a multitude of proteins to the plasma membrane. Our understanding of how these proteins accomplish their tasks at their target location is hampered by insufficient information about their detailed interactions with all membrane components. Current experimental methods suffer from low resolution or unphysiological conditions, whereas atomistic molecular dynamics simulations cannot access the time scales relevant for lipid rearrangements or protein re-orientation. As a solution, we have developed a multi-scale molecular dynamics approach that combines a systematic screening of possible binding orientations by coarse-grained simulations with subsequent atomistic simulations of the most relevant states. After excellent benchmark results for the well-studied PH-domain of PLC- $\delta 1$, we applied our method to study the activation process of focal adhesion kinase (FAK). PIP2 strongly modulated the orientations of different FAK fragments. The FERM domain interacted with PIP2 through the same residues that engage in binding the catalytic domain. Our results suggest a stabilization of FAK's dissociated state by PIP2-FERM interactions, which is consistent with previous observations and can inspire new experiments.

Posters

– 11. Computational biophysics –

P-361

Directing membrane pore formation in MD simulations using an embedded mechanical ‘gadget’

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Stalk and pore formation are two well-known membrane topological changes that are required for a host of cellular processes. Their atomistic mechanisms and energetic budgets, however, are still not fully known due to the fact that the transitions are rare (large activation energies), localized (involve very few lipid and solvent molecules), and transient (quick, once activated). These factors make the transitions notoriously hard to probe both experimentally and with simulations. In particular, most simulation schemes to enhance sampling near the transition barriers suffer from severe hysteresis effects.

Here we present a simulation method to reversibly direct pore formation and closure using a membrane embedded mechanical ‘gadget’. This ‘pore gadget’ mimics the function of a lipid scramblase, but it can be adjusted to open, close or restrain a nascent pore, thereby permitting exhaustive sampling of topological intermediates states such as indentations and water wires spanning the hydrophobic slab. We can also recover an unbiased (lipid+solvent only) free energy landscape for poration by reweighting an ensemble of gadget+membrane simulations. Preliminary tests also suggest that a ‘stalk gadget’ can direct hydrophobic stalk formation in a similar manner.

P-363

Investigation of peptide binding affinity and thermal stability of Human Leukocyte Antigens (HLAs)

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Peptide immunogenicity is defined as the ability of a peptide to stimulate Cytotoxic T Lymphocytes (CTLs) mediated immune response, hence T cell activation is a key event in the adaptive immune response. Therefore, understanding the characteristic of Human Leukocyte Antigens (HLAs) dynamics is crucial to gain insights on the mechanism of T-cell activation. The peptide immunogenicity is related to peptide binding affinity and stability of peptide-HLA complex. In this study, in order to predict the peptide binding affinity and to investigate the stability of peptide-HLA complexes, various computational techniques have been employed. Initially, computational dockings were applied to model the peptide-HLA complexes using FlexPepDock. 50 ns molecular dynamics simulations were conducted on these models via GROMACS 5.0.1 and peptide binding affinities were predicted using g_mmpbsa GROMACS tool to investigate the thermal stability of the modeled peptide-HLA complexes. The key residues playing a role in binding stability are determined, which can be used to enlighten the relationship between stability and affinity of these complex molecules in addition to developing insights on the mechanism of peptide immunogenicity.

P-362

Amphiphilic cyclodextrins: how long MD simulations give a full picture of membrane insertionS. Buchoux¹, A. L. Furlan¹, V. Bonnet², C. Sarazin¹¹Génie Enzymatique et Cellulaire FRE CNRS 3580, Université de Picardie Jules Verne, 80000 Amiens Cedex, France;²Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources UMR CNRS 7378, Université Picardie Jules Verne, 80000 Amiens Cedex, France

Glycerolipidyl-cyclodextrins (G β CD) exhibit amphiphilic properties that lead to self-aggregation and cell membrane affinity; making them potential drug nanovectors. While their aggregation and interaction with lipids can be studied by e.g. light scattering or ²H NMR, experimental characterization of the dynamics involved during membrane insertion is hardly possible.

By using several- μ s-long MD simulations of DOCD, a dioleoylated G β CD, with model membranes (DMPC), we were able to describe completely the penetration of the amphiphilic cyclodextrins inside the lipid bilayer:

At first, the DOCD molecules self-assemble to form a small aggregate that will enter the lipid membrane. The insertion mechanism is a two-step process: first the whole aggregate penetrate the bilayer before being entirely dissolved in the lipid environment, leaving single DOCD molecules in the hydrophobic core. From the lipid perspective, the membrane first undergoes a disorganization followed by an increase of the lipid chain order as it can be followed by the lipid order parameter S_{CD}. Experimentally, only this final more-ordered state is observable but MD simulations allow to get the full picture.

P-364

Markov state models of protein aggregationM. Carballo-Pacheco^{1,2}, U. Sengupta^{1,2}, B. Strodel^{1,3}¹Research Center Juelich, Germany; ²RWTH Aachen University, Germany; ³Heinrich Heine University Duesseldorf, Germany

Protein aggregation into highly structured amyloids fibrils is associated with diseases such as Alzheimer's and Parkinson's. The toxic species in these diseases is not the fibrils but smaller, more disordered oligomers. Because of the transient nature of these oligomers, it is often hard to characterise them experimentally. Hence, simulations of protein aggregation are customarily used to provide mechanistic details of the process. Here, we present an extension of Markov state models to study protein aggregation, which allows us to precisely identify the aggregation intermediates. In such a way, we can understand the intra- and intermolecular re-ordering that oligomers follow at relevant in vivo concentrations. We apply our methodology to the amyloid-forming peptide KFFE.

Posters

– 11. Computational biophysics –

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Interaction of LCAT enzyme with lipid surfaces and apolipoprotein A-I derived peptides

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The lecithin cholesterol acyltransferase (LCAT) enzyme catalyses the formation of cholesteryl esters which is detrimental for the formation of mature spherical high density particles (HDL) which in turn play a crucial role in the reverse cholesterol transport (RCT). Given the importance of efficient RCT in decreasing the development of coronary heart disease (CHD), it would be beneficial to understand how LCAT and apolipoprotein A-I (apoA-I), the superior activator of LCAT, are mediating the enzymatic reaction in unison at the atomistic level. Here, we have studied how LCAT interacts with a lipid bilayer and apoA-I derived amphiphilic peptides through atomistic molecular dynamics simulations and surface sensitive biophysical methods. We show that LCAT attaches on the lipid bilayer surfaces through a hydrophobic patch located at the previously suggested membrane interacting region, but also through the lid region of LCAT. Further, we demonstrate how phospholipids and cholesterol enter the active site of LCAT and how apoA-I may facilitate this. The results presented here could prove to be valuable in the designing of novel molecular therapies for LCAT deficiencies and CHD.

P-367

Improved understanding of protein dynamics via energy landscape sampling, analysis, and comparison

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This talk will revisit three fundamental problems for energy landscapes of biomolecules.

First, a hybrid exploration algorithm combining basin hopping and rapidly exploring random trees will be sketched, yielding an enhanced exploration of complex landscapes.

Second, an algorithm to analyze a collection of conformations each endowed with a potential energy will be presented; we shall use it to identify so-called persistent local minima, their attraction basins, and connexions across saddles. Finally, an algorithm to compare two (sampled) energy landscapes will be outlined. The strategy, based on optimal transportation theory, consists of computing a least cost mapping between the basins, compatible with the transition paths known on both landscapes.

Illustrations on various systems whose (frustrated) landscapes have been exhaustively studied in the literature will be presented.

The software implementing the algorithms discussed is made available to the community in the Structural bioinformatics Library (<http://sbl.inria.fr>).

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P-366

Multi-scale modelling of large biomolecular complexes

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As a fundamental component of the plant-digestion machinery from bacterial cells, the cellulosome is a complex biological nanomachine composed of multiple proteins connected by flexible linker regions. It provides a key target for computation-design re-engineering due to its key potential role in development of environmentally-friendly processes to produce biofuels and fine chemicals from the breakdown of biomass waste.

We are using quantum mechanically-derived molecular dynamics force fields together with coarse grained models and free energy simulations to guide the development of so-called "designer cellulosome" capable of fast and cost-effective breakdown of feedstock waste for biofuel production. Such a complex design problem not only challenges supercomputing capabilities but also exploits the latest developments in out-of-equilibrium simulation methods, accelerated sampling, and analysis methodologies to extract relevant information from very large datasets.

Our data shows that naturally-occurring glycosylation serves to keep the self-assembling units in their active forms and at optimum distances from each other. We also show that the units can be rationally re-engineered into states of lower and higher mechanostability, as very recently validated by our experimental collaborators using force microscopy and spectroscopy measurements.

P-368

Refining molecular dynamics simulations of RNA using solution NMR data

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RNA structure and dynamics play a fundamental role in non-coding RNAs and significantly affect functions such as gene expression inhibition, splicing and catalysis. Molecular dynamics is a computational tool that can be in principle used to investigate RNA structure and dynamics at atomistic resolution. However, its capability to predict and explain experimental data is limited by the accuracy of the employed potential energy functions, also known as force fields. Recent works have shown that state-of-the-art force fields could predict unphysical conformations that are not in agreement with experiments. The emerging strategy to overcome these limitations is to complement molecular dynamics with experimental data included as restraints. Solution NMR data are particularly useful since they provide averages over the conformations explored on the experimental time scale and ultimately give access to RNA dynamics. We here propose a scheme based on the maximum entropy principle to combine bulk experiments with molecular dynamics simulations explicitly taking into account experimental errors. The method is applied to a set of nucleosides and dinucleotides in a chemically-consistent manner and suggests a new paradigm for force field refinement.

Posters

– 11. Computational biophysics –

P-369

Ab initio calculation of structural and elastic properties of Mg_2Sn and Mg_2Pb compounds

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University of Larbi Tébessi, Laboratoire de Physique Appliquée et Théorique LPAT, Algeria

We present first principles studies of structural and elastic properties of antiferromagnetic compounds Mg_2Sn and Mg_2Pb . All the calculations are carried out using the plane wave pseudopotential method within the density functional theory (DFT) in the local-density approximation. The equilibrium lattice parameters are obtained by minimizing the energy with respect to volume. The elastic properties are calculated such as the elastic constants C_{ij} and the Young modulus E , shear modulus G and Poisson ratio $[1B2?]$ by using the Voigt-Reuss-Hill averaging.

P-371

Prediction of the anti-apoptosis and pro-apoptosis proteins based on domain and motif information

Y.-L. Chen, J.-T. Zhao

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Apoptosis proteins can be classified to anti-apoptosis and pro-apoptosis which have the opposite regulation effect on apoptosis. So the research of the advanced structure of the anti-apoptosis and pro-apoptosis proteins, as well as the identification based on structural information can help us to understand the proteins function. In this paper, we constructed the anti-apoptosis and pro-apoptosis protein datasets. Each protein has annotation information of the domain or motif from the UniProtKB. Based on amino acid sequence components information, hydropaths component, blocks information, n-terminal sequence components information, segmented single peptide information, position-specific scoring matrix, domain and motif information, the anti-apoptosis and pro-apoptosis proteins are predicted by the SVM algorithm in Jackknife test. Based on the results, the predictive capability of each single characteristic parameters and fusion characteristic parameters are analyzed and discussed respectively.

Keywords: apoptosis, domain, SVM algorithm**Acknowledgements**

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P-370

Plasma membrane localization dynamics of the filovirus matrix protein VP40

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Filoviruses such as Ebola and Marburg are highly contagious and cause hemorrhagic fever. They are lipid-enveloped viruses that obtain their lipid coat from the plasma membrane of the host cell they infect during the budding process. The major Filovirus matrix protein VP40 is trafficked to and localized at the inner leaflet of the plasma membrane. Once associated with the membrane, VP40 dimers undergo structural rearrangements and oligomerization into filaments to form the viral matrix that provides the scaffolding for the new virus particle. Therefore, trafficking of the dimeric form of VP40 protein and its stabilization at the lower leaflet of the plasma membrane is an important step for the matrix formation and viral budding. We used molecular dynamics simulations to investigate the lipid-protein interactions of VP40 dimers and hexamers at the plasma membrane. We quantified lipid-lipid self-clustering by calculating the fractional interaction matrix and found that the VP40 hexamer significantly enhances the PIP_2 clustering. Our MD simulations in the presence and absence of membrane interactions show that lipid-binding can affect the VP40 conformations. These computational studies support recent experimental data and provide new insights into the mechanisms by which VP40 assembles at the plasma membrane inner leaflet and forms new virus-like particles.

P-372

Proteins at liquid interfaces: insights from molecular simulation

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The adsorption of proteins onto liquid interfaces is central to a number of biological processes and applications in biotechnology. Adsorption of proteins onto such interfaces is typically associated with changes in protein conformation, often with a consequent loss of function. Obtaining a detailed molecular description of this structural change is a key step to understanding protein structure and function in complex, interfacial environments.

In this presentation I will describe some recent work aimed at investigating adsorption and conformational change of proteins at liquid interfaces. Specifically the adsorption of *hydrophobins*, amphiphilic, fungal proteins onto oil-water interfaces will be discussed. Using coarse-grain simulations the adsorption strength of two of these proteins was found and related to their surface structure. The conformations adopted by two myoglobin-derived peptides will also be discussed. Both peptides adopt similar, compact conformations in bulk solution and readily adsorb onto the air-water interface. They adopt significantly different conformations at interfaces, with one of these adopting a flat, extended conformation these different interfacial conformations can be related to their emulsification behaviour.

Posters

– 11. Computational biophysics –

P-373

Decoding and tailoring cooperative DNA recognition at atomic resolutionV. Cojocaru^{1,2}

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Transcription factors are proteins that bind to DNA regulatory regions in cells to control gene transcription. Usually, they cooperate to recognize complex DNA motifs, enhancing or modifying their DNA sequence specificity. Hence, an additional layer of regulation is established that is key to cellular function. It is often not understood how structural features and dynamics modulate the cooperative binding to DNA. Here, I am presenting our progress in establishing these mechanisms for OCT4 and SOX2, two essential master regulators of stem cell identity. Using a series of molecular simulations and free energy calculations we found that their cooperativity is modulated by an interplay of protein-protein interactions and DNA-mediated structural signals induced by the individual binding events. Moreover, we predicted and validated a series of mutations that modified the cellular function of SOX2 and of a related SOX factor by changing their ability to cooperate with OCT4. These studies pioneered the application of molecular simulations to engineer transcriptional circuitries for cell fate transitions. Currently, we strive to decode the mechanisms by which the cooperative DNA recognition modifies the DNA sequence specificity in the absence of protein-protein interactions.

P-375

Parallel folding pathways of UCH-L1 - protein with Gordian knot – differ in topology of intermediateP. Dabrowski-Tumanski^{1,2}, Y. Zhao^{1,3}, S. Niewieczeral¹, J. I. Sulkowska^{1,2}

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Human ubiquitin C-terminal hydrolase, UCH-L1, is a Gordian (5₂) knotted protein, and one of the most abundant brain proteins [1]. Its folding is known to occur via complex mechanism, consisting of at least two parallel pathways [2]. Both pathways are two-step processes, with the intermediate state prone to oligomerization, leading to neurodegenerative diseases. However, nor the health-endangering intermediate states, nor the folding pathways were studied in detail.

Here we combine the strength of coarse grained dynamics and mathematical knot theory to bridge the gap in characterization of the UCH-L1 folding pathways. In particular, we show that the parallel pathways differ in the topology of their intermediate states. We study the probabilities of each folding pathway, mean folding times of each pathway and their dependency on temperature. To validate our findings, we correlate them with experimental evidences. Finally, we suggest new experiments which may confirm our proposed model of UCH-L1 folding, solving finally the mystery of self-tying of 5₂-knotted proteins.

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P-374

Assessment of the interactions of phthalates with the human cytochromes P450D. Craciun¹, D. Dascalu^{2,3}, A. Isvoran^{2,3}

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Phthalates are used in the manufacture of plastics, solvents, personal care products and medical devices. As a consequence, they may enter into the human body by inspiration and/or by orally absorption. Published data concerning the effects of phthalates on animal and human expose are quite controversial. Within this study we produce the ADME-Tox profiles of the most common phthalates. The biggest part of them reveals at least a low degree of toxicity when they are orally absorbed and produce irritation when inhaled. Our computational studies also predict that phthalates have inhibitory potential on the human cytochromes P450 (CYP) that are involved in xenobiotics metabolism: CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Within this study we assess these possible interactions by molecular docking. Our results indicate that almost all of the considered phthalates are able to bind to the catalytic site of at least one of CYP enzymes. These interactions could alter the physiological, pathological, pharmacological and toxicological effects of the phthalates in human organism.

P-376

Mass spectrometry based modelling of macromolecular assemblies

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Small Heat-Shock Proteins (sHSP) are present in all kingdoms of life, and form assemblies spanning a continuum of structures from mono- to polydisperse, with variable architecture. These can bind unfolding proteins, preventing their potentially harmful denaturation. Since sHSP oligomers can bind several proteins simultaneously, ensembles of >>100 sHSP:target stoichiometries are often observed. Mass Spectrometry (MS) is one of the few techniques able to separate these complexes and assess them individually. By exploiting MS structural data, we generate plausible polyhedral models for sHSPs, and describe a possible binding mode to their targets.

This work led to the development of novel computational tools of general applicability. These include methods to predict cross-linking while accounting for both cross-linker and protein flexibility, to calculate the collision cross-section of electron density maps, and to arrange molecules according to arbitrary topologies. These methods are all integrated in BiobOx, a Python package allowing the analysis and manipulation of molecular structures and ensembles at an atomistic, super coarse-grain, or electron density level.

Overall, we show that MS data can be successfully exploited for the modelling of protein assemblies.

Posters

– 11. Computational biophysics –

P-377

Towards realistic models of lung surfactant - MD simulations with improved water and ion force field

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Lung surfactant lines the gas-exchange interface in the lungs and reduces the surface tension at the air-liquid boundary to minimize the work of breathing. The lung surfactant consists mainly of lipids with a small amount of proteins. It forms a monolayer at the air-water interface connected to bilayer reservoirs in the sub-phase. The composition of the pulmonary surfactant and the border conditions of normal human breathing are relevant to characterize the interfacial behavior of pulmonary layers.

In this work, we aim at constructing a realistic model of lung surfactant to be used in atomistic molecular dynamics simulations. At first, we focus on lipid components of the pulmonary surfactant. We base our approach on recent advances in force field development for lipids, ions, and water. We also include realistic, lipidomics-based lipid mixtures in the model. We consider lipid monolayers composed of DPPC, POPC, POPG and cholesterol under physiological temperature and concentration of ions. On computational side, we employ Slipids force field combined with charge-scaled ions and new OPC model of water. This approach is able to properly reproduce lipid-lipid interactions, correctly account for ion-lipid interaction and also reproduce the experimental surface tension of the system.

P-379

Conformational states of Kras in its active and inactive formB. Dudás¹, F. Merzel², D. Perahia³, E. Balog⁴

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RAS proteins are in the forefront of cancer research. Despite the intensive efforts of the past decades to date no efficient RAS inhibitor has been discovered yet. It is difficult to target Ras directly as the oncogenic mutants are locked in the active (GTP-bound) conformation. There is a high intracellular concentration of GTP and the protein has a great affinity for it while Ras has only an inherently low GTPase activity. While there is a large amount of experimental data (e.g. more than 100 X-ray structures), our view of RAS structure and mechanism through which it is regulated is mainly based on static structures, and overlooks conformational dynamics and allosteric effects. While an increasing number of simulation data are emerging, there is an acute need for simulation methods that can efficiently map the conformational surface of KRAS. The goal of our research is to map the conformational space of the GDP bound inactive and the GTP bound active states, to detect the interactions between the HVR (hyper variable region) and the catalytic domain and to discover similarities and differences between the two activation states.

P-378

RRCRank: a fusion method using rank strategy for residue-residue contacts predictionQ. Dong¹, X. Hu²

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Motivation: Protein residue-residue contacts is important in protein structure study, especially in de novo protein structure prediction. In this study, we present a novel fusion method using rank strategy for protein residue-residue contacts prediction, which formulates the contacts prediction problem as a ranking problem. The proposed method extracts features from ensemble machine-learning classifiers and correlated mutations methods firstly, and then predicts the probabilities of residue pairs to be contacts based on learning-to-rank.

Results: The proposed fusion method, RRCRank, is evaluated on the CASP11 dataset and 40 CASP12 targets which are released recently. Compared with the other well-developed methods, the RRCRank has improved performances for all three categories of contacts, especially for short-range and medium-range contacts. We also implement traditional classification and regression strategies to predict contacts, the result shows that the proposed ranking strategy has superiority for contacts prediction compared with traditional strategies. Furthermore, we compare the RRCRank with four leading methods which participated in CASP11, the RRCRank has a comparable performance and outperforms three methods in most evaluation metrics on CASP11 all targets dataset.

P-380

How does membrane composition modulate cholesterol carrier protein NPC2?G. Enkavi^{1,2}, H. Mikkolainen², B. Güngör^{3,4}, E. Ikonen^{3,4}, I. Vattulainen^{1,2}

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Niemann-Pick Protein C2 (NPC2) is a small soluble protein, which facilitates the endosomal/lysosomal cholesterol efflux, a vital step in cholesterol metabolism. Mutations in NPC2 cause Niemann-Pick C disease, in which lipid accumulation causes neuronal degeneration and early death. Specific lipids found in the lysosome/late endosome affect the efficiency of NPC2-mediated cholesterol transport, but the molecular mechanism of this activity modulation remains elusive. We performed atomistic molecular dynamics simulations and free energy calculations to investigate the effect of relevant lipids on NPC2-membrane binding. We characterize a mechanism for membrane association and two distinct membrane binding modes of NPC2: a “cholesterol-exchange mode” and an “idle mode”. We systematically show that i) anionic lipids are necessary and sufficient for unspecific membrane association; ii) a unique anionic lysosomal/endosomal lipid, bis(monoacylglycero)phosphate (BMP), however, is required for the “cholesterol exchange mode”; and iii) sphingomyelin (SM) counteracts BMP by favoring the “idle mode”. Our findings suggest that BMP and SM modulate NPC2-mediated cholesterol transport by changing how NPC2 binds the membrane.

Posters

– 11. Computational biophysics –

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Molecular dynamics simulations of cationic polymers

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Polyethylenimine (PEI) is an essential component of PEI-DNA complexes, being extensively employed in a variety of non-viral gene delivery systems. The charge distribution along the PEI chains is widely considered to be responsible for the effectiveness of PEI-DNA condensation.

We parametrized an additive CHARMM force field, derived from high-quality ab initio calculations, with the partial atomic charges, bonds, angles, and dihedrals specifically optimized for PEI. The developed force field was used to investigate the dynamical behavior of PEI chains by extensive molecular dynamics simulations. Synthetic parameters, such as gyration radius, end-to-end distance, persistence length, radial distribution functions, coordination numbers, and diffusion coefficients were analyzed. Our force field leads to qualitatively more rigid PEI chains than those reported in the literature and we find diffusion coefficients in excellent agreement with experimental calculations.

P-383

The prediction of long-range enhancer-promoter interactions

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The long-range interactions between enhancers and promoters play a critical role in the regulation of gene expression. In this study, based on the interactions dataset from Carbon Copy Chromosome Capture Conformation (5C) experiments for four human cell lines, an AdaBoost algorithm and ensemble classification strategy for the identification of enhancer-promoter (E-P) interactions in the dataset constructed by Roy et al. (2015) were proposed by using the extracted features of transcription factors (TFs), histone modifications (HMs), DNase I, DNA methylation and RNA-seq in Gm12878, H1hesc, Hela and K562 cell lines. We obtained the area under the precision-recall curve (AUPR) values with about 5%-9% higher than the previous results for Gm12878, H1hesc, Hela and K562 cell lines, respectively, in 10-fold cross-validation. The results indicated that the E-P interactions have cell line-specific tendency. Some potential key regulatory elements are found.

Acknowledgements

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P-382

An engineered peptide toxin analogue with improved Kv1.3 selectivity displays reduced flexibility

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Anurotoxin (AnTx), a 35-amino-acid scorpion toxin, is a high affinity blocker of voltage-gated K⁺ ion channels. Since Kv1.3 channels play a key role in the activation of T lymphocytes, which are key mediators of immune responses, direct manipulation of T lymphocytes via Kv1.3 inhibition has been proposed as an effective strategy of achieving immune suppression. Thus, selective Kv1.3 blockers hold a great potential in the therapy of certain autoimmune diseases. AnTx inhibits Kv1.3, but also blocks Kv1.2 expressed in other cells of the body with similar potency. Single and double mutants of AnTx showed improved selectivity for Kv1.3 over Kv1.2 while kept high affinity for Kv1.3. The results obtained from the structures based on NMR and from MD simulations suggested that the restricted conformational space of the double substituted toxin compared to the flexible wild-type AnTx is an important determinant of toxin selectivity [1,2]. The findings may provide foundation for the possibility of designing additional, even more selective toxins targeting various ion channels.

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P-384

The HYDRO software for solution properties of bio-macromolecules and nanoparticles

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The HYDRO suite [1] comprises a variety of tools for predicting and analyzing hydrodynamic and other solution properties of biomacromolecules and nanoparticles, both rigid and flexible, such as the well-known programs HYDROPRO [2] for predicting properties from atomic- or residue-level structures, and HYDRO++, for general bead models. Other tools connect hydrodynamic determinations with orthogonal techniques like NMR, cryo-EM or SAXS/SANS. The SIMUFLEX [3] package covers coarse-grained, mesoscale prediction of properties and dynamic simulation of flexible structures with conformational variability, like intrinsically disordered proteins and complex RNAs. Some recent extensions and improvements in this suite will be also described.

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Posters

– 11. Computational biophysics –

P-385

Effects of the L483Y mutation on the ligand binding domain of the AMPA receptor

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Ionotropic glutamate receptors are crucial for many important neurological processes such as memory and learning. In this study a particular mutant of the AMPA receptor, L483Y as well as other mutants such as L483F, L483A and L483T are investigated. The L483Y mutant is physiologically characterised through its ability to blocking desensitization of the AMPA receptor.

The mechanistic understanding of this behaviour is investigated using a combination of classical molecular dynamics and umbrella sampling. The impact of the L483Y mutation on the GluA2 ligand binding domain and our results support that this mutation may stabilize the ligand binding domain interface through a cation- π interaction previously reported. However, our data also seems to highlight significant stabilization of a salt bridge crossing the dimer interface.

P-387

Multiscale simulations of partially disordered systemsC. Globisch¹, C. Dalgicdir², M. Sayar³, C. Peter¹

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Coarse grained (CG) models are widely used to study peptide self-assembly and nanostructure formation. One of the recurrent challenges in CG modeling is the problem of limited transferability. A crucial question for peptides is whether a model reproduces the molecule's conformational response to a change in its molecular environment. Examples are conformational transitions between a rather disordered and an ordered state upon a change in pH value or due to the presence of a soft apolar/polar interface. To handle such transitions CG models mostly utilize auxiliary interactions to aid secondary structure formation. Such interactions take care of properties of the real system that are per se lost in the coarse graining process such as dihedral-angle correlations along the backbone or backbone hydrogen bonding. Since the CG models are designed to emphasize certain conformational propensities they may destroy the ability of the model to respond to stimuli and environment changes. This points out how important it is to investigate whether they impede transferability. To analyze such processes in combined atomistic/CG manner a common characterization by utilizing dimensionality reduction methods can be used to compare the conformational free energy landscapes at both resolutions.

P-386

Dynamic network communication in large proteinsT. Ghane¹, M. Bagherpoor Helabad¹, K. F. Santos², E. Absmeier², M. C. Wahl^{2,3}, P. Imhof¹

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Different regions/residues in a protein can communicate by sending or receiving signals. These signals can be transferred in the form of conformational changes. Signals can move from one residue to the other one by passing from other residues, each one modifying conformation of the next in some way. Designing a consistent framework of allostery in proteins can pave more general understanding of proteins function. In this study we apply dynamical network analysis to the trajectories of MD simulations in two proteins, Cco and Brr2, to get insight into the molecular mechanism of allostery in these two proteins. Analysis of MD simulations reveals protonation state dependent conformational dynamics and hydrogen-bond dynamics of key channel residues in Cco [1], and communication between N cassette and C cassette in Brr2.

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P-388

Hierarchical TMD dynamics provides a rationale for presentation of the APP ϵ -sites to γ -secretase

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Alzheimer's disease (AD) is characterized by accumulation of toxic β -amyloid ($A\beta$) in the brain and neuronal death. $A\beta$ peptides of different lengths are produced by stepwise proteolytic cleavage within the transmembrane domain (TMD) of the amyloid precursor protein (APP) by γ -secretase. $A\beta$ toxicity is related to fragment length, which correlates with the site of initial cleavage at $\epsilon 48$ or $\epsilon 49$. Mutations located upstream of the ϵ -sites shift production towards the longer, aggregation-prone $A\beta 42$, associated with early-onset familial AD (FAD). Using molecular dynamics simulations of wild-type and FAD mutants, we gain mechanistic insights into the interplay between substrate TMD's conformational dynamics and presentation of its ϵ -sites. We show that a shift in initial cleavage from $\epsilon 49$ to $\epsilon 48$ is not a consequence of mutant-induced locally enhanced helix unwinding of the scissile bonds. Rather, increased fluctuations upstream of the ϵ -site interfere with the native organization of backbone flexibility. This allows movement of rigid helix segments with respect to each other around more flexible regions, which can act as hinges. FADs shift the location of identified hinge-sites and, as a consequence, the related exposure of the ϵ -sites to the active site of γ -secretase.

Posters

– 11. Computational biophysics –

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Exploring the $\text{Ca}^{2+}/\text{Na}^{+}$ selectivity of NaChBac channel through Molecular Dynamics simulationsC. Guardiani¹, M. Rodger², I. Khovanov¹¹School of Engineering, University of Warwick, UK;²Department of Chemistry, University of Warwick, UK

NaChBac is a puzzling member of the family of bacterial voltage gated sodium channels. In fact, despite its Na^{+} selectivity, it features the same Selectivity Filter (SF) motif as calcium channels. In this work we compared sodium and calcium permeation mechanisms by means of unbiased and metadynamics simulations. When the channel is simulated in a NaCl solution, the SF is occupied by no less than two ions and access of a third ion is frequently observed. By contrast, only a single Ca^{2+} ion can access the SF. While the axial Potential of Mean Force (PMF) of Na^{+} shows 4 minima separated by modest barriers, Ca^{2+} PMF shows 3 minima, the two innermost being separated by a high barrier that prevents further advancement of the ion. Our simulations thus show that even if a Ca^{2+} ion can access the SF, it remains stuck inside and can not fully permeate the channel. We propose two possible explanations of this pattern. First of all, the free energy of binding of Ca^{2+} to the SF is higher than that of Na^{+} so that calcium is unlikely to spontaneously leave this binding site. Moreover, a $\text{Ca}^{2+}/\text{Ca}^{2+}$ knock-on mechanism is prevented by the strong electrostatic repulsion that the resident ion exerts on the potentially incoming second ion.

P-391

Re-engineering the mechanostability of cellulosomal proteins via site-directed mutagenesis

M. Gunnoo, P.-A. Cazade, D. Thompson

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The degradation of cellulosic biomass into fermentable sugars is a pivotal step for the conversion into biofuels. One of the most efficient types of cellulase systems in nature involves an extracellular multi-enzymatic complex named the cellulosome. One way to enhance the efficiency of the cellulosome for mass production of biofuel is to re-engineer the complex in order to improve the mechanostability of its domains.

Techniques such as Single Molecule Force Spectroscopy (SMFS) have been used to study the mechanical stability of cellulosomal proteins. However, in order to access atomic-resolution and also understand the interatomic interactions, molecular modelling is necessary.

In this study, steered molecular dynamics is used to quantify the intermolecular contacts that determine the mechanical stability of the family 3 Carbohydrate Binding Module (CBM3) and cohesin 7 from CipA scaffoldin. Simulations identified candidates for site-directed mutagenesis which coupled with rational design of a loop region that produced the required low-stability module, in the case of CBM3. While for the cohesin, proposed mutants improved its mechanical stability, crucial for the industrial application of cellulosomes for the production of fuels, chemicals and pharmaceuticals.

P-390

Thermostability of dockerin-cohesin pairs from three cellulosome species with MD simulations

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Since the production of biofuel relies on the transformation of simple sugars into ethanol, worldwide effort is currently focused on identifying and consequently engineering enzyme complexes which are capable of efficiently degrading organic wastes into fermentable sugars. Naturally occurring cellulosome complex is described as a highly efficient enzymatic nanomachine that binds and degrades the cell wall.

The cellulosome architecture consists of a non-catalytic “scaffoldin” subunit and two complementary recognition modules termed “dockerin” and “cohesin” that together serve to integrate the various enzymes into the complex. Thermostable cellulolytic enzymes are particularly attractive candidates for biomass deconstruction. Their resistance and robustness to high temperature (above 50–55°C) can allow faster and more effective reaction. However, the specific high affinity cohesin-dockerin interaction, a fundamental component of the cellulosome assembly, is reduced at high temperatures.

The aim of this project is to simulate cohesin-dockerin pairs of three different species at elevated temperatures and consequently, attempt to tune their thermal stability through single site mutations.

P-392

Redox-driven proton pumping in respiratory complex I : a molecular dynamics simulation studyO. Haapanen¹, V. Sharma^{1,2}¹Department of Physics, University of Helsinki, Finland;²Institute of Biotechnology, University of Helsinki, Finland

Respiratory complex I (NADH:ubiquinone oxidoreductase) is the first electron acceptor in the mitochondrial respiratory chain. It reduces the ubiquinone molecule by electrons derived from NADH, and couples it to the pumping of protons across the inner mitochondrial membrane¹. Complex I function is essential for the synthesis of ATP in cells. Point mutations in complex I have been associated with various neurodegenerative disorders. Thus, any mechanistic understanding of enzyme function would open new avenues for drug development. To understand the molecular mechanism of complex I, we have constructed a state-of-the-art model system in full lipid-solvent environment comprising about a million atoms², and performed atomistic classical molecular dynamics simulations in multiple redox and protonation states of the enzyme. Our results reveal rapid hydration of both the hydrophobic and the hydrophilic subunits of the enzyme, and provide novel insights into how redox reactions at the active site may be coupled to the long-ranged proton pumping ca. 200 Å away. The mechanistic hypotheses based on simulation data can be easily tested with the experiments.

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Posters

– 11. Computational biophysics –

P-393

A Prion-like mechanism in Amyotrophic Lateral Sclerosis

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A prion-like mechanism has been developed to explain the observed promotion of amyloid aggregation caused by conversion of structurally intact SOD1 to a misfolded form. Superoxide dismutase [Cu-Zn], or SOD1, is a homo-dimeric protein that functions as an antioxidant by scavenging for superoxide. The misfolding and aggregation of SOD1 is linked to inherited, or familial, amyotrophic lateral sclerosis (FALS), a progressive and fatal neurodegenerative disease. Aberrant SOD1 folding has also been strongly implicated in disease causation for sporadic ALS, or SALS, which accounts for ~90% of ALS cases. Studies have found that mutant, misfolded SOD1 can convert wtSOD1 in a prion-like fashion, and that misfolded wtSOD1 can be propagated by release and uptake of protein aggregates. Here it is demonstrated that enervating the SOD1 electrostatic loop can lead to an experimentally observed gain of interaction (GOI) responsible for the formation of SOD1 amyloid-like filaments. This enervation is caused in turn by the formation of transient, non-obligate oligomers between pathogenic SOD1 mutants and wt SOD1.

P-395

Charge transfer in photolyases

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This poster will represent the results of theoretical studies describing charge transfer in the enzyme Photolyase. The main task of this light sensitive enzyme is the binding and repair of damaged DNA. This function involves several electron transfer (ET) steps, which activate a non-covalent bond FAD cofactor and carry an electron to the damage in the DNA. Quantum dynamics simulations of ET use a QM/MM approach in which the traveling charge and some selected sites e.g. amino acids are calculated with a QM-method (DFTB) and coupled to the rest of the system being propagated with MM methods.

Reaction times for several ET steps in a photolyase PhrB of *Agrobacterium fabrum* were calculated and compared to other proteins of this family. The same simulations were performed for mutations of PhrB which indicate the correlation between specific amino acids and the ET.

The product of these ET processes are systems with separated charges which force the protein and the environment to adopt. This leads to several processes e.g. proton transfer reactions or structural changes in the protein which will also be described and compared to experimental studies.

P-394

Platform for a computational integrated analysis of structure and mutation data on SLC transporters
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Membrane transporter proteins play an important role in transportation of nutrients and waste, in maintenance of homeostasis, and in signal transduction. Solute carrier (SLC) transporter is the second largest superfamily of membrane proteins with at least 386 genes in human genome. SLC transporters recognize a wide variety of substrates such as amino acids, peptides, saccharides, ions, neurotransmitters, lipids, hormones and related materials. Exogenous substances such as drugs and environmental hormones are also taken up by cells through SLC transporters. Despite the apparent importance for the substrate transport, the three-dimensional structures, evolutionary history, and relation to disease of SLC transporters have not been well investigated. We, therefore, developed a new tool to assist the search for the relationship between structural characteristics and pathogenic mutations on SLC transporters. We built a relational database by connecting annotations, sequence characteristics, conformational features, mutations, and related disease information of human SLC transporters. As a result, we found that the chance of finding pathogenic mutations was significantly higher on transmembrane and ordered regions than that of benign mutations.

P-396

Dynamics and energetics of intrinsic tubulin bending: Novel implications for microtubule assembly

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Microtubules (MTs) are key components of the cytoskeleton and play a central role in cell division. They undergo stochastic switching between phases of growth and shrinkage driven by GTP hydrolysis in tubulin dimers. MT assembly is associated with tubulin having a straight conformation, while during disassembly it adopts a curved conformation that is incompatible with the MT geometry. The mechanism by which GTP binding and hydrolysis control this process is still poorly understood. In particular, two models of MT assembly are being controversially discussed. Whereas in the allosteric model GTP binding directly promotes tubulin straightening, tubulin is curved regardless of the bound nucleotide in the lattice model. Here, we characterize the conformational space and energetics of free tubulin in solution using intensive atomistic molecular dynamics (MD) and free energy calculations. Unexpectedly, we find that both GTP- and GDP-tubulin adopt either a curved or an intermediate curved conformation. Furthermore, GTP binding increases the intrinsic dimer flexibility rather than promotes its straightening. Our results suggest a consensus model that combines the notion of the permanently curved conformation of free tubulin with the allosteric effect of GTP binding.

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– 11. Computational biophysics –

P-397

Oligomerization pathway of A β fragments by the Hamiltonian replica-permutation methodS. G. Itoh^{1,2}, H. Okumura^{1,2}¹Institute for Molecular Science, Japan; ²The Graduate University for Advanced Studies, Japan

The amyloid- β peptides (A β) form amyloid fibrils which are associated with Alzheimer's disease. It is necessary to clarify the oligomerization process of A β in order to understand the amyloid fibril formation process and to find a remedy for Alzheimer's disease. To investigate the oligomerization process of A β , we applied the Hamiltonian replica-permutation method (HRPM) to A β fragments, A β (29-42) peptides, in explicit water solvent. A β (29-42) consists of the residues 29 to 42, which correspond to the transmembrane domain of A β . The length of A β after residue 29 is a critical determinant of the amyloid formation rate. Moreover, this fragment forms amyloid fibrils by itself.

HRPM combines the advantages of RPM and the Hamiltonian replica-exchange method (HREM). RPM is a better alternative to REM. In RPM, temperature permutations among more than two replicas are performed with the Suwa-Todo algorithm. In HREM, by exchanging the parameters that are related only to limited degrees of freedom, the number of replicas can be decreased in comparison with REM.

I will introduce HRPM and show the oligomerization process of A β (29-42) in my presentation.

P-399

CEED: A novel biophysical approach to altering protein stability and enzyme activityH. Jones¹, V. L. Arcus², S. A. Wells¹, M. van der Kamp³, F. Macmillan⁴, C. R. Pudney¹

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Enzyme thermal stability can potentially be engineered via site-directed mutagenesis. This is desirable not only for the industrial use of enzymes, but also to study fundamental concepts in the biophysics of protein folding and enzymology. We are developing a novel protein engineering approach based on understanding intramolecular protein dynamics. The CEED (Computational Enzyme Engineering with Dynamics) approach predicts and detects intramolecular protein dynamics and seeks to identify mutants to optimise the rigidity of the protein whilst retaining the native flexibility of the active site. The process involves an initial screen using all atom flexible motion calculations from a known structure using FIRST (Floppy Inclusions and Rigid Substructure Topography) that will be validated with molecular dynamics simulations. We have tested our approach using monoamine oxidase (MAO-B), and preliminary results indicate a 3°C increase in melting temperature (T_m) for one point mutation. These findings suggest CEED will be a powerful biophysical tool for protein and enzyme engineering, and will contribute to the ongoing debate regarding the role of protein dynamics in enzyme catalysed reactions. Additional studies on MAO-B have also elucidated a potential new mechanism for the enzyme.

P-398

Multiscale simulation study of penta peptide aggregation: From atomistic details to a mechanistic interpretationA. Jain¹, N. K. Mishra², C. Globisch¹, S. Verma², C. Peter¹

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Development of new well ordered, functional biomaterials based on the underlying principal of self assembly has immense application in nanotechnology, nanomedicine and tissue engineering. Peptide based nano-materials are not only biocompatible but also their properties can be altered easily by slight changes in environmental conditions and/or side-chains of amino-acids. Herein, we report a multiscale simulation study of penta peptides that exhibit very different morphologies upon altering a single amino acid. Atomistic simulations identified governing factors that lead to specific peptide morphology such as peptide flexibility vs rigidity, role of dimerization and the partitioning of hydrophobic side chains. The study was extended with coarse grained simulations. That allowed general conclusions about the mechanistic origin of the different morphologies. Our systematic study with different backbone beads and supportive pseudo dihedral angles illustrate the importance of very careful and delicate selection of coarse grained parameters to reproduce the chemical and structural properties of the system.

P-400

Impact of cell size on efficacy of single-cell C4 photosynthesisI. Juric¹, V. Gonzalez-Perez¹, J. Hibberd², G. Edwards³, N. Burroughs¹

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C4 photosynthesis is the name for a biochemical pump present in some higher plants, that boosts the carbon assimilation efficiency by concentrating atmospheric CO₂ at the place where carbon fixation occurs. The C4 mechanism typically develops in tandem with Kranz anatomy, a leaf-level architectural feature where multiple cell walls within a leaf function as diffusion barriers, preventing CO₂ leakage. In some C4 lineages (e.g. Bienertia family) the C4 mechanism instead operates on the level of individual mesophyll cells. It is not clear how these plants accomplish high photosynthetic efficiency without adopting Kranz anatomy. By modelling the processes of diffusion, capture, and release of carbon dioxide and oxygen inside a typical Bienertia mesophyll cell geometry, we show that a spatial separation, as low as 10 μ m, between the entry and exit points of a C4 pathway, can, on its own, provide enough diffusive resistance to sustain a viable C4 pathway at 20°C with a CO₂ leakage less than 35% - a value comparable to Kranz anatomy plants. This critical separation, corresponding to a cell diameter of 50 μ m, is consistent with the observed range where Bienertia's mesophyll cells start to develop their characteristic mature anatomy.

Posters

– 11. Computational biophysics –

P-401

Influence of polymorphic conformations of DSS1 on its binding with BRCA2

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DSS1 is an intrinsically disordered protein, which is widely conserved during evolution. Owing to its intrinsic disorder, DSS1 is capable of binding different partners and exhibits polymorphic conformations. In human, it plays an important role in stability of BRCA2, and stable DSS1-BRCA2 complex is an essential component of DNA repair mechanism. Depletion of DSS1 is known to be associated with human breast and ovarian cancer. Although the structural basis of DSS1-BRCA2 interaction is well understood, the role of polymorphic conformations of DSS1 in binding of BRCA2 is still elusive. In this study, we simulated different configurations of native and mutated DSS1 in bound and unbound states. This study explores the influence of conformational polymorphism of DSS1 on its binding with BRCA2. Besides, we have also shown how mutations can affect the conformational dynamics of DSS1. Our analysis shows the secondary structure of DSS1 is transient and is influenced by the binding of BRCA2. The effect of polymorphic conformations on the interaction energies is also discussed. Our findings establish the significant role of polymorphic conformations of DSS1 in binding of BRCA2, and provide a framework for understanding the dynamics of DSS1-BRCA2 interactions.

P-403

gmfit : an approximated 3D shape for atomic model and density map using Gaussian mixture model

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Recently, a Cryo-electron microscopy provides 3D density maps of the biomolecular assemblies with middle to high resolution (3–10 Å). To interpret the density map, an atomic model should be built by fitting known 3D subunit structures or finding secondary structural elements. To reduce a computation time for the modeling, we introduced a 3D Gaussian mixture model (GMM) for approximated 3D shape of a density map and an atomic model. The GMM is made of a weighted sum of 10 – 40 Gaussian functions. A new expectation-maximization algorithm is developed to convert a 3D map or atoms into GMM, regarding each grid or atom as small one Gaussian function. After converting to GMMs, two maps or atomic models can be quickly superimposed using the gmfit program. We opened the web service “pairwise gmfit” for one-vs-one fitting of two 3D density map or atomic model. This service is linked to the “Omokage search”, which is a service to search the global shape similarity of biological assemblies, in both the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB). The program gmfit is also able to perform many-vs-one fitting using several types of spatial restraints, such as symmetry and proximity. Other potential applications of GMM are also discussed.

P-402

Excess sodium dramatically alters binding preference between oxoanions and cationic amino acids

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Polyatomic oxoanions are often present in biological and environmental systems, but the roles they play are not yet fully understood. Molecular simulations using classical, atomistic models can potentially shed light into processes involving these ions, provided that model parameters exist. We present parameters for the interactions of HSO_4^- , RSO_4^- , RSO_3^- , H_2PO_4^- , R_2PO_4^- , CH_3COO^- , SO_4^{2-} , HPO_4^{2-} and RPO_4^{2-} with TIP3P water, as well as with Na^+ , NH_4^+ and RNH_3^+ (R=alkyl groups). This parameter set is internally consistent, enabling the study of ion specific effects involving these species. We find that the presence of high concentration (150 mM) of sodium may greatly alter the preference of anions for the cationic amino acid lysine: anions with intrinsically large binding affinities for lysine may, in the presence of excess sodium, demonstrate much lower binding affinity than expected. These results shed light into the molecular scale origin of binding affinities between anionic polymers and cationic proteins reported from experiment: the experimentally determined binding affinities do not reflect the *intrinsic* affinity of the anions for the cationic amino acids, but the effective affinity that results from competition with sodium.

P-404

Theoretical study of protein complex systems by using a coarse-grained model and its development

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Effective interactions between protein molecules play key roles in many protein functions. In our previous study, we have constructed the coarse grained potential for hydrophobic amino acid residues in an aqueous solution and reproduced the complex structure of GCN4-pLI tetramer by the coarse grained simulation [1].

The purpose of this study is to construct the coarse grained potential for charged amino acid residues and polar amino acid residues in an aqueous solution. The coarse grained potential function is constructed to reproduce the effective interaction between two amino acid side chain analogues in an explicit water solvent estimated by the aaMD simulations. Then, the Langevin dynamics simulation with the coarse grained potential is performed to reproduce the protein complex structure.

References

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Posters

– 11. Computational biophysics –

P-405

Incorporation of protein-ligand interactions into elastic network model

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Elastic network model (ENM) is extended to render protein-ligand interactions atomistically, while treating the interactions within the protein chain at the coarse-grained level of alpha-carbons. This approach is based on our previously introduced mixed-resolution model for obtaining atomistic details on supramolecular systems (Kurkcuglu et al., *Biophys. J.*, 97: 1178, 2009). In the model under consideration, the interaction strengths are not uniform over the network as opposed to the classical ENM model. Their values are given by a function of the distance between interacting pairs. We assessed the success of this model over the classical approach using a dataset of diverse proteins including monomers, dimers and tetramers of various sizes. We determined the parameter set that leads to high correlations between experimental and theoretical mean-square fluctuations for protein residues and/or ligand atoms. Incorporation of the atomistic ligand in the complexes yields better correlations compared to the ones obtained from classical ENM results. This extended ENM model is suitable to observe the changes in vibrational frequencies and mode shapes upon complexation.

P-407

Statistical (mechanical) model for exploring protein sequence subspace

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The multiple sequence alignment (MSA) of a protein family provides a wealth of information in terms of the conservation pattern of amino acid residues not only at each alignment site but also between distant sites. In order to statistically model the MSA incorporating both short-range and long-range correlations as well as insertions, I have derived a lattice gas model of the MSA based on the principle of maximum entropy [1]. The partition function, obtained by the transfer matrix method with a mean-field approximation, accounts for all possible alignments with all possible sequences. The model parameters for short-range and long-range interactions were determined by a self-consistent condition and by a Gaussian approximation, respectively. Using this model with and without long-range interactions, I analyzed the globin and V-set domains by increasing the "temperature" and by "mutating" a site. The correlations between residue conservation and various measures of the system's stability indicate that the long-range interactions make the conservation pattern more specific to the structure, and increasingly stabilize better conserved residues. Application to protein sequence design is also discussed.

[1] A. R. Kinjo (2016) *Biophysics and Physicobiology* **13**:45–62. DOI:10.2142/biophysico.13.0_45

P-406

Plasma induced pore formation in model cell membranes: Molecular dynamics simulation studies

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The oxidization or peroxidation of phospholipids, which may cause serious damages on cellular membrane, has recently attracted much attention from various research fields. Especially, it was reported that the dielectric barrier discharge (DBD) plasma treatment can induce apoptosis, damage in DNA, and dysfunction of mitochondria in mammalian cancer cells. Experimental observations indicate that 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipids can be oxidized into fragmented lipid molecules by the DBD plasma treatment. Hence, in this study, we have built up various model membrane systems using the oxidized molecules along with intact DOPC lipids. Performing both atomistic and coarse-grained molecular dynamics simulations of these model systems, we have observed that the presence of the damaged species in DOPC membranes considerably affects both structural and dynamical properties such as compressibility modulus, tail angle distribution, density profile, order parameter, and self-diffusion coefficients. Especially, it was observed that the oxidized lipids escape from the membrane and induce the water pore formation in the membrane. Interestingly, the pore formation is more closely correlated with the lipid assembly than with the lipid escape.

P-408

Intermolecular interactions in the activation of Two Pore Channels

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Two Pore Channels (TPCs) are intracellular ion channels that are widely expressed in eukaryotic cells. Depending on the host cell, they are involved in diverse processes like the cellular cation and pH homeostasis, Ebola virus infection and cancer cell migration. The gating mechanism and regulation of these channels are therefore of strong interest. It was shown that TPC1 of *Arabidopsis thaliana* gets activated in a Ca^{2+} and voltage dependent manner. Furthermore, patch-clamp experiments with wild type and truncated variants demonstrated that the C-terminus of AtTPC1 is an indispensable player for channel activity. In contrast, the homologous TPC2 of humans is gated open upon addition of phosphoinositides (PI(3,5)P₂), however, the exact binding site and the relation to channel activation are unknown. To investigate the mode of channel activation of AtTPC1 and hTPC2 we combined experimental techniques and molecular dynamics simulations at the coarse-grained and atomistic level. Results demonstrated that AtTPC1 subunits interact via their C-terminal regions, and PI(3,5)P₂ lipids tend to bind to predominately positively charged sub-regions of hTPC2. Further experiments will show if these homologues share common features in the gating mechanism.

Posters

– 11. Computational biophysics –

P-409**Transmembrane protein-induced membrane curvature**

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The local curvature of cellular membranes can function as a sorting mechanism for transmembrane proteins, e.g. by accumulation in regions of matching spontaneous curvature (SC), as shown recently for potassium channel KvAP and water-pore AQP0 by Aimon *et al.* [Aimon (2008), *Dev. Cell*, **28**(2), 212–218]. However, the direction of the reported SC as well as the molecular background could not be addressed experimentally yet. Using coarse-grained and atomistic molecular dynamics simulations, we analyzed the levels of spontaneously induced curvature for the homologous potassium channel Kv 1.2/2.1 Chimera (KvChim) and AQP0 when embedded in unrestrained POPC lipid nano-discs. Coarse-grained results are in excellent agreement with the experiments, at values of -0.036 nm^{-1} and 0.019 nm^{-1} induced by KvChim and AQP0, respectively. Furthermore, the direction of curvature can be retrieved directly from the simulations. Atomistic simulations of both systems show SC comparable to the coarse-grained results, and allow for detailed investigation of its origin, especially in terms of protein-lipid interactions. Here, uneven distribution and organization of POPC lipids at the interface of KvChim establishes a basal negative curvature, which is then further modified by the dynamics of the protein.

P-411**Efficient molecular dynamics simulation of linker histone ubiquitylation**

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Many DNA-based processes is controlled by histone modifications such as methylation, acetylation and modification with ubiquitin (Ub). It is suggested, that mono-ubiquitylation of linker histone (H1) might play a role in controlling gene expression, in DNA repair, in the maintenance of genomic stability, in antiviral protection, as well as its participation in the histone code, but remains one of the least understood epigenetic modifications. In experimental studies, it was shown, that after ubiquitylation, H1 and Ub come together and form a compact structure, but a question of the dynamics of this process remains. Molecular dynamics (MD) simulations of H1 which was ubiquitylated at different sites allow investigation of the possible influence of Ub (by shielding parts of H1 surface) on the strength or kinetics of H1/DNA binding. But already for this two domain system, the timescale problem impedes a straightforward application of MD simulations. Application of advance sampling methods is required. Recently we proposed the expansion scheme, which uses dimensionality reduction to sample efficiently a phase space of intrinsically disordered peptides. We apply this method to simulate ubiquitylated H1 and compare an influence of different ubiquitylation sites.

P-410**Empirical determination of nearest-neighbor amide I coupling constants in a helical peptide**

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Conformation-dependent couplings among Amide I local mode oscillators give rise to Amide I vibrational band contours that reflect the three-dimensional structures of peptides and proteins. Non-nearest-neighbor coupling is often competently modeled via the transition dipole coupling (TDC) mechanism. However, the close proximity of nearest-neighbor (i.e., sequentially adjacent) amide groups requires models that realistically account for the charge distribution of the amide group. In this poster, we report direct observation of the sign and magnitude of Amide I nearest neighbor coupling via linear infrared spectroscopy of isotopomers of a short helical peptide enriched with carbon-13. Comparison of linear infrared spectra of doubly-enriched isotopomers with spectra of singly-labeled analogues allows for the computation of coupling constants. Our results are compared to quantum mechanical and electrostatic map calculations.

P-412**Structural communication in cancer-related transcription factors**

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Little is known about conformational changes induced at distal sites in cancer-related transcription factors (TF) [1–3]. We are focusing on the characterization of TF dynamics by a combined approach that integrates atomistic classical and enhanced sampling simulations, graph theory and cross-validation with NMR data. To relate these properties to protein function, we studied both the free and DNA-bound forms of wild-type, phosphorylated and mutated variants. The interaction with DNA not only stabilizes the conformations of the DNA-binding loops but also strengthens pre-existing paths in the free proteins for long-range communication to interfaces for cofactor recruitment. The interaction with DNA promotes conformations of the distal regions that are a minor population of the free ensemble, altering the preferences for certain classes of partners and thus influencing their signaling pathways.

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Posters

– 11. Computational biophysics –

P-413

Hydrocarbon stapling of peptides confers drug-like properties: A mechanistic computational studyJ. Li^{1,2}, C. Verma², R. Beuerman¹¹Singapore Eye Research Institute, Singapore;²Bioinformatics Institute, A*STAR, Singapore

Stapled peptides are a promising class of therapeutics that possesses many druggable properties. Here we use multi-scale molecular dynamics (MD) simulations to study the effect of hydrocarbon stapling on the solution helicity, membrane permeability and binding mechanism of an anti-cancer stapled peptide PM2. Hamiltonian replica exchange MD (HREMD) simulations revealed that the hydrocarbon stapling enhances PM2 helicity via a combination of constraint effect and hydrophobic effect. Using coarse-grained MD simulations, we show that PM2 experiences much lower free energy penalties than the unstapled peptide during translocation across the membrane, suggesting hydrocarbon stapling promotes membrane penetration. Moreover, hydrocarbon stapling also modulates the binding mechanism. PM2 maintains the helical conformation during approaching to the binding pocket of the receptor MDM2, while the unstapled peptide undergoes conformational changes from unstructured conformations to helical conformation during binding. Furthermore, the hydrocarbon staple chain also directly interacts with the hydrophobic pocket of MDM2, resulting in enhanced binding affinity. The findings of this study provide insightful guidance for the design of novel stapled peptide therapeutics.

P-415

Molecular determinants of the influenza fusion peptide's activityD. Lousa¹, A. R. T. Pinto², B. L. Victor^{1,4}, A. Laio³, A. S. Veiga², M. A. R. B. Castanho², C. M. Soares¹

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There is an urgent need to develop vaccines and drugs against influenza virus (IV) and a promising strategy to combat this virus is to inactivate the fusion process between the viral and host membranes, which is mediated by the protein hemagglutinin (HA). During this process, the N-terminal region of HA, known as fusion peptide (FP), inserts into the host membrane. Although it is known that the FP plays a crucial role in the fusion process, the molecular effect of the peptide remains unclear.

We used a combination of state-of-the-art experimental and simulation techniques to analyse the WT influenza FP and four mutants[1]. The energy landscape of the peptides was analysed using bias-exchange metadynamics simulations. This allowed us to compare the conformational properties of the WT and mutant peptides in a model membrane. To complement the simulation results, fluorescence based methods were used to analyse the peptides' partition coefficient in model membranes, and their ability to promote lipid-mixing was analysed by a (FRET)-based assay. The results obtained in this study contribute to a better understanding of the role of the influenza FP in the fusion process.

1. Lousa D., et al., **2016**, *Scientific Reports*, 6:28099

P-414

Nanoparticle builder: new software for preparing nanoparticles for molecular dynamics simulationsF. Lolicato^{1,2}, J. Akola^{2,3}, H. Martinez-Searaa^{2,4}, I. Vattulainen^{1,2}

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Molecular dynamics simulations are a useful approach to study the interaction between monolayer-protected metal nanoparticles and lipid membrane systems. Simulations allow one to test in detail how different shapes, terminal groups, and side chain lengths modulate nanoparticle properties, thereby providing major added value for experimental work. Given this, simulations have considerable potential to cut the costs associated with nanoparticle synthesis and testing. However, as for simulations, the progress in the field is slowed down by difficulties to prepare nanoparticle structures and related files in an efficient manner. Here we present a new software package that automates the nanoparticle building process. The 3D molecular structures and the related simulation files generated by the software are compatible with the most common molecular dynamics simulation packages, such as GROMACS, NAMD, and LAMMPS. Further, the topologies used in describing nanoparticles are compatible with the force fields used commonly in the field, including in particular CHARMM36, Slipids, OPLS2-AA, GROMOS54a7, and Martini.

P-416

Mechanism of coiled-coil deformation under tension strongly depends on the pull speed

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Dimeric coiled coils are formed by two alpha-helices wrapped around each other, forming a super helix. These folding motifs play important roles in various intracellular regulation processes as well as in membrane fusion. They can also potentially be used as force sensors in biological systems, giving insight into the spatial distribution of mechanical forces with a resolution of a few nanometers. To investigate this possibility, we examined the mechanical response of *de novo* designed coiled-coils with different lengths, using Steered Molecular Dynamics simulations. At sufficiently slow pulling speeds, the pulling force is largely independent of either the initial coiled-coil length or of the contact length between the two helices. At those speeds, the coiled-coil stretches via unfolding-assisted sliding. Coiled-coil stretching via helix unfolding, observed in several prior studies, is only present at the higher pulling speeds, which are more distant from the biologically relevant regime.

Posters

– 11. Computational biophysics –

P-417

Orientation of lutein and zeaxanthin molecules in the phospholipid bilayer

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Carotenoids are the second largest group of plant pigments. They are part of the photosynthetic complex, responsible for light harvesting and energy dissipation. They also play a role as membrane photoprotectors. Carotenoids are synthesised only by plants and bacteria, while animals have to receive them from food. Animals need carotenoids as light filters and membrane protectors. Decreased consumption of lutein is associated with increased risk of eye diseases, especially age related macular degeneration.

Lutein and zeaxanthin are two main carotenoids of retina. Although they have similar structures, their localisation within the retina is different – concentration of zeaxanthin is higher in the middle and that of lutein is higher in the outer regions of the retina. Moreover, model studies indicate that their orientation in the lipid bilayer is different. To verify this, computational methods were used to analyse the orientation of lutein and zeaxanthin in the phosphatidylcholine bilayer. For lutein, both transmembrane and horizontal orientations were observed. In contrast, for zeaxanthin only transmembrane orientation was observed. Orientation of the carotenoid molecules may affect the fluidity of the membrane as well as their interactions with potential partners.

P-419

Computational approaches to the development of new Beta1 Integrin antagonists and ADMET evaluation

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Integrins are cell adhesion receptors that transmit bidirectional signals across the plasma membrane. They are non-covalently linked heterodimeric molecules consisting of one α and one β subunit. Integrins play an important role in inflammation and cancer, due to their properties in cell proliferation processes. Three integrin inhibitors were selected to be docked with three receptors: $\alpha 4\beta 1$, $\alpha 5\beta 1$ and $\alpha V\beta 1$ and their ADMET properties were estimated. The aim of this study is to develop new integrin antagonists with pharmacological potential. The chosen ligands (BIO1211, BIO5192 and TCS2314) were built using Avogadro. Molecular Docking was done with Vina. Rachel was used for the design of the new compounds using ZINC database. ADMET predictions were performed using the programs ClogP/CMR, FAF-Drugs3, ProTox, and OSIRIS Property Explorer. The lowest calculated energy was -9.2 kcal/mol for $\alpha 4\beta 1$ and BIO5192. Rachel generated more than 200 new BIO5192 derivatives. The scores ranged the first tenth ligands from 9.08 to 8.45. The results from ProTox showed low toxicity of the ligands. These results show that BIO5192 is promising as a lead compound for the development of new integrin inhibitors. This is a filtering approach to improve drug discovery and development processes.

P-418

Double-stranded DNA and RNA under constant stretching forces: insights from molecular dynamics

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Multiple biological processes involve the stretching of nucleic acids. Stretching forces induce local changes in the molecule structure, inhibiting or promoting the binding of proteins, which ultimately affects their functionality. Understanding how a force induces changes in the structure of nucleic acids at the atomic level is a challenge. Here we use all-atom microsecond-long molecular dynamics to simulate the structure of dsDNA and dsRNA subjected to stretching forces up to 20 pN. We determine without any prior assumption all the elastic constants of dsDNA and dsRNA which we found to be in excellent agreement with the experimental data. Furthermore, we provide for the first time an atomistic explanation for three striking differences in the mechanical response of these two molecules, i.e.: the three-fold softer stretching constant obtained for dsRNA, the opposite twist-stretch coupling and its non-trivial force dependence. The lower dsRNA stretching resistance is linked with its more open helix-structure. The opposite twist-stretch coupling of both molecules is traced down to the slide base pair parameter. More interestingly, we found that the force evolution of the slide parameter is highly non-trivial and dependent on the NA in question.

P-420

A computational approach to self-assembly of new antimicrobial peptides

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Antimicrobial resistance has been listed as one of the greatest threats to human health, worsened by the lack of new antibiotics. Recently engineered liposomes have been showed to sequester pore-forming toxins promoting antimicrobial activity and were proven to be suitable for drug delivery. However the mechanism of such self assembly is still unknown, as well as the characteristics that most enhance the antimicrobial activity.

The project first aims at testing how simulation techniques describe the antimicrobial and self-assembly processes and compare with experimental data: integrating different simulations hopefully lead to establish the chemico-physical properties that favour those mechanisms. The subsequent goal is to explore amino acids mutations on given peptides and new building scaffolds to enhance such properties to design more powerful antimicrobial peptides and organize them in structures suitable for drug delivery.

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– 11. Computational biophysics –

P-421

Investigating the behavior of β 2-m wild type and its amyloidogenic variants with MD simulations
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β 2-microglobulin (β 2-m) is a protein responsible for the amyloidosis that affects patients suffering from renal failure, producing fibrillar deposits on osteoarticular tissues. Following recent advances, we investigated β 2-m wild type and its mutants in the most likely first steps of fibrillation process that remain unclear to researchers.

β 2-m WT and amyloidogenic mutants Δ N6 and D76N were studied in protein-protein interactions by full atomistic MD simulations. With the aid of various analysis tools, including Essential Dynamics, we monitored the behavior of the interface patches and the internal structural rearrangements. For Δ N6 we found a “zipping” mechanism of closure driven by formation of salt-bridges, further investigated by Temperature Replica Exchange MD.

Currently, the focus is on Pro32 cis-trans isomerization, one of the amyloidosis triggering factor. We apply enhanced sampling methods on the monomeric forms of β 2-m WT, D76N amyloidogenic mutant and W60G aggregation-resistant mutant to study the Free Energy Landscape of each species, determining the role of mutations on the stability of the two isomers and on the height of the free-energy barrier that separate them.

P-423

Non-adiabatic QM/MM simulations of cyclobutane thymine dimer formation in DNAJ. I. Mendieta-Moreno^{1,2}, D. G. Trabada¹, J. Mendieta², J. P. Lewis³, P. Gomez-Puertas², J. Ortega¹¹Universidad Autónoma de Madrid, Spain; ²Centro de Biología Molecular Severo Ochoa, Spain; ³West Virginia University, USA

The absorption of ultraviolet radiation by DNA may result in harmful genetic lesions that affect DNA replication and transcription, ultimately causing mutations, cancer and/or cell death. Here we analyze, using hybrid quantum mechanics / molecular mechanics techniques and non-adiabatic molecular dynamics, the role of the double helix structure on the photostability of DNA. We study the most abundant DNA photolesion, the cyclobutane thymine dimer, and find that DNA presents a free energy barrier between native configurations and reactive conformations leading to the photolesion. Our results show that most of the photo-excited reactive conformations return to native DNA configurations after an ultrafast non-radiative decay to the ground state. These two factors explain the low probability (1.5×10^{-4}) of photolesion formation per absorbed photon. These properties of the DNA molecule have probably played an important role in the early stages of life evolution when the surface of Earth was under intense ultraviolet radiation.

Jesús I. Mendieta-Moreno et al, *J. Phys. Chem. Letters* 2016, 7 (21), 4391–4397

P-422

ECCLipids17: adapting atomistic lipid models to correct cation-membrane interactionsJ. Melcr, S. H. Ollila, K. Baxova, P. Jungwirth, H. M. Seara
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Classical molecular dynamics simulations give detailed information about membrane structure and dynamics. However, there is still a room for improvements in current force fields – it is known from the literature, that the binding of ions, especially cations, to phospholipid membranes is overestimated in all classical models [Ollila 2016]. We suggest that the membrane-ion interactions can be corrected by including implicit electronic polarizability into the lipid models through the electrostatic continuum correction (ECC) [Leonatyev 2010], which was already applied to monovalent and divalent ions yielding models that feature correct ion pairing [Kohagen, 2015]. Using the electrometer concept [Ollila 2016, Seelig 1987], our preliminary pilot simulations point out that our hypothesis is correct and ECC is indeed a missing important contribution in current classical lipid models. In order to create a lipid model that reproduces relevant biological membrane properties, however, we have to adapt the whole force field to the new ECC paradigm. Results from such a development that leads to a lipid model with correct cation-membrane interactions, “ECCLipids”, will be presented. The project is carried out within the open collaboration platform NMRlipids (<http://nmrlipids.blogspot.fi>).

P-424

Robust simulation workflows for alchemical binding free energy calculationsA. S. J. S. Mey, J. Juaréz Jiménez, J. Michel
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Robustly predicting free energies of binding of small/drug like molecules to proteins still remains a challenge in computational chemistry despite the increase in available computational power and vast algorithmic improvements. In particular, the implementation of reproducible workflows for alchemical free energy methods relying on molecular dynamics is often lacking. Here we will present a high-throughput workflow pipeline for reliably setting-up, running alchemical binding free energy simulations and analysing the obtained data. This pipeline makes use of available software tools such as FESetup for protein and ligand preparation and Sire SOMD for simulation, but also newly developed analysis tools that allow for an adaptive and iterative simulation-analysis-refine simulation protocol that can be run on both small GPU clusters as well as cloud computing facilities such as Amazon Web services. In order to demonstrate the capabilities of this simulation analysis pipeline the Farnesoid X receptor will serve as an example protein system with a large experimental dataset of binding affinities, made available through the Drug Design Data (D3R) grand challenge 2.

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– 11. Computational biophysics –

P-425

Steered molecular dynamics simulation for studying ATP-analogues into P2X2 receptor binding site

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P2X receptors constitute a family of ligand-gated ion channels that open in response to the binding of extracellular adenosine 5'-triphosphate (ATP). P2X2 receptor is widely expressed in mammalian tissues, where they are involved in a range of processes like nociception and enteric neurotransmission. Hence, elucidating the ligand interaction at P2X receptors has become an important target for the development of rational drug design.

The main goal of this work is to characterize ATP analogues g-[2-azidoethyl]-ATP and EDA-ATP specific interaction into P2X2 receptor binding site. For this purpose, using molecular docking, molecular dynamics simulations and steered molecular dynamics we have studied the thermodynamics processes associated to the analogue binding and correlated with single molecule analysis obtained by atomic force microscopy. These results have provided novel insights into the molecular pharmacology of P2X receptors. Funded by ICM-P10-035F and DPI-CONICYT 20140080.

P-427

In silico validation of NorA homology model from S. aureus using FEP and Metadynamics

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NorA membrane transporter of *S. aureus* is responsible for inducing MDR by pumping out of the cell a wide range of antibiotics, especially fluoroquinolones. Since the structure of the NorA model is not solved we constructed a structural model with I-TASSER program in order to develop novel inhibitors.

Known NorA phenylquinoline inhibitors were used to identify the putative RBS by molecular docking. Molecular dynamics results revealed clear and distinct interactions patterns for actives and inactives. While hydrophobic interactions and a h-bond made with the quinoline nucleus were more or less conserved between actives and inactives, a highly specific h-bond was established only with the side chain of the actives.

Alchemical transformation active ligands into inactive ones impacted the relative binding free energy ($\Delta\Delta G$) by 3–7 kcal/mol. Relative binding free energy differences between the actives were between 0.5–1.5 kcal/mol and < 1 kcal/mol for the inactives.

Metadynamics simulation with the same ligands within the binding site revealed results that are very much comparable with FEP simulations, allowing differentiation of actives based on ΔG . Based on the results, we are confident that the validated model can be used to identify novel NorA efflux pump inhibitors.

P-426

Theoretical studies on dynamics of electron carriers in photosynthesis by a coarse-grained model

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Plastocyanin(PC) is one of type I copper proteins which have one copper ion in their active site. The function of the type I copper protein is electron transfer between proteins. In photosynthesis, PC transfers one electron from cytochrome f in cytochrome b₆f complex to P700 in Photosystem I by diffusing in the lumen side of the thylakoid membrane. In order to investigate both association process and diffusion process of PC, coarse-grained simulation is efficient to simulate large system sizes on long time scale. In our pervious study, we have developed coarse-grained intermolecular interaction including the hydrophobic interaction.

In this study, We present a simple coarse-grained model for describing intermolecular interaction between proteins in solvent to investigate the association, self-assembly behaviour, and binding modes with complex configurations. The new expression of the hydrophobic potential is presented in similar way to the concept of molecular-crowding effects. We discuss the association process of type I copper proteins Plastocyanin-Cytochrome f by using our simple coarse-grained model in relation to the binding modes between interacting proteins and to the free energy landscape of the complex.

P-428

Acceleration of QM/MM molecular dynamics for metal-containing large biomoleculesH. Nishizawa¹, H. Okumura^{1,2}

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Molecular dynamics (MD) has been employed for investigating the properties of biomolecular systems. In several cases, MD simulations are performed by the molecular mechanical (MM) approach because a long time step is required for obtaining sufficient sampling. However, the MM approach cannot accurately describe a biomolecular system including a metal ion. On the other hand, metal ions play a crucial role in the living body. A quantum mechanical/molecular mechanical (QM/MM) approach based on the density-functional tight-binding (DFTB) theory is a useful tool for analyzing such a chemical reaction system in detail. However, when QM/MM calculations are performed under periodic boundary conditions (PBCs), there is one problem. Because the Coulomb term decays very slowly, its computational cost increases.

In this study, an efficient QM/MM method by the combination of the DFTB/MM method with the particle mesh Ewald (PME) method under PBC is developed. Because the Fock matrix, which is required in the DFTB calculation, is analytically obtained by the present method, the Coulomb energy is accurately and rapidly computed, i.e., our method is 166.5 times faster than the conventional method without loss of accuracy.

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– 11. Computational biophysics –

P-429

Computationally-guided design of high affinity binders from intrinsically disordered regions (IDRs)

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Over 60% of the proteins encoded in the human genome contain intrinsically disordered regions (IDR). Many IDRs are involved in protein-protein interactions (PPI), which usually cover small surface area, and varying degrees of folding on site upon binding. XPA is a key protein involved in nucleotide excision repair (NER). XPA recruits the ERCC1-XPF endonuclease onto the 5' side of the DNA damage site. Binding of the XPA to ERCC1 is essential in this process and involves a disordered region of XPA. The peptide XPA67-80 has been shown to inhibit the XPA-ERCC1. As ERCC1 overexpression is an indicator of resistance to platinum-based chemotherapeutics, we are developing a set of ERCC1-specific high affinity peptide and peptidomimetic binders, with a design based on the molecular features essential for the recognition of human XPA₆₇₋₈₀ by ERCC1. In this work, we show how molecular dynamics can be used to identify these features. We discuss the conformational ensemble of XPA from 3 species that carry mutations that could enhance the peptide binding affinity, and the binding of a synthetic derivative of XPA₆₇₋₈₀ under development in our lab, whose structural design is based on the identification of the molecular recognition features (MoRFs) of the naturally occurring XPA₆₇₋₈₀.

P-431

Structural difference between two ends of A β amyloid fibril revealed by molecular dynamicsH. Okumura^{1,2}, S. G. Itoh^{1,2}¹Institute for Molecular Science, Japan; ²The Graduate University for Advanced Studies, Japan

A β amyloid fibrils, which are related to Alzheimer's disease, have a cross- β structure consisting of two β -sheets: β 1 and β 2. The A β peptides are thought to be serially arranged in the same molecular conformation along the fibril axis. However, to understand the amyloid extension mechanism, we must understand the amyloid fibril structure and fluctuation at the fibril end, which has not been revealed to date. Here, we reveal these features by all-atom molecular dynamics (MD) simulations of A β 42 and A β 40 fibrils in explicit water. The structure and fluctuation were observed to differ between the two ends. At the even end, the A β peptide always took a closed form wherein β 1 and β 2 were closely spaced. The A β peptide fluctuated more at the odd end and took an open form wherein the two β -sheets were well separated. The differences are attributed to the stronger β -sheet formation by the β 1 exposed at the even end than the β 2 exposed at the odd end. Along with the small fluctuations at the even end, these results explain why the fibril extends from one end only, as observed in experiments. Our MD results agree well with recent observations by high-speed atomic force microscopy.

P-430

Physiological model of the mechanism of action of ciprofloxacin on *E. coli*

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Combating the emergence of antibiotic resistance requires better understanding of antibiotic mechanism of action as well as the mechanisms by which resistance evolves. The antibiotic ciprofloxacin traps the DNA-gyrase complex, inhibits DNA replication, and can cause DNA double-strand breaks. However, a physiological model to explain bacterial cell death and growth-inhibition curves in the presence of ciprofloxacin is still lacking. Here we develop a simple model of replication fork inhibition and DNA double strand breakage by ciprofloxacin. Using stochastic simulations we reproduce the shapes of experimentally measured growth-inhibition curves and predict their growth-medium dependence.

P-432

Accelerating lipid and lipid-protein molecular simulations by a factor of two using virtual sites

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The accessible time scales of Molecular Dynamics simulations of biomolecules are limited by an integration timestep of 2 femtoseconds (fs), which is required to resolve fast bonded degrees of freedom of bonds, angle and dihedrals involving hydrogen atoms. For proteins, the timestep can be increased to 5 fs using a virtual sites (VS) approach, where the bonded degrees of motion freedom of H atoms are eliminated by making the H atoms massless while retaining their VdW and electrostatic interactions. We have extended the VS formalism to all lipids of the CHARMM 36 force field, thus accelerating the performance of lipid and lipid-protein simulations by a factor of two or more, without losing atomic resolution of H atoms.

Posters

– 11. Computational biophysics –

P-433

Interaction between a short DNA oligonucleotide and urea in the light of Kirkwood-Buff theory

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In nature, a wide range of biological processes, such as transcription termination or intermolecular binding, is dependent on the formation of specific DNA secondary and tertiary structures. These structures can be both stabilized or destabilized by the osmolytes, coexisting with the nucleic acids in the cellular environment. In our study, we investigate a short 7-nucleotide DNA hairpin with the sequence d(GCGAAGC) in the presence of varying concentrations of the osmolyte urea.

The interaction between DNA and urea in unbiased molecular dynamics simulations has been analysed according to Kirkwood-Buff theory. We implemented the local/bulk partitioning model, complemented by the analysis of preferential hydration and preferential interaction coefficients, to get insight into the distribution of the cosolute in the vicinity of the DNA oligonucleotide. The free energy landscape of unfolding has been approached via Metadynamics upon the addition of a bias potential. This study allows us to get a more comprehensive understanding of the stability of the DNA structures in the presence of urea.

P-435

Mechanistic study of cofactor B12-dependent enzymes

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Adenosylcobalamin (AdoCbl, coenzyme B12) is nature's radical repository designed to catalyse challenging chemical reactions by generating reactive free radical intermediates. The biophysical basis of B12-dependent catalysis has long intrigued biochemist and chemists alike. In addition to the effects of ground-state destabilisation, strain hypothesis and electrostatic factor, experimental studies have also highlighted the importance of coupling between protein dynamics and the chemical steps. Hence, the complexity of the radical-based reaction chemistry entangled by the diversity of the protein conformation landscape present a technical challenge for computational simulations of B12-dependent enzymes. Using a variety of computational approaches, including the combined QM/MM and metadynamics methods, our studies have provided evidence of how protein dynamics on a variety of time scale is used to control the generation of radical intermediates in D-ornithine 4,5-aminomutase and ethanolamine ammonia lyase. Our computational study reveals fundamental biophysical strategies employed by B12-dependent enzymes in the control of radical catalysis.

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P-434

Investigation of intrinsic dynamics and allosteric coupling in human β 2-adrenergic receptorO. Ozcan¹, C. Ozgur¹, P. Doruker², E. D. Akten³

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This study is based on a molecular model of human β 2-adrenergic receptor (β 2-AR), which incorporates the mostly neglected intracellular loop 3 (ICL3). To emphasize the effect of ICL3 on receptor's intrinsic dynamics, MD simulations were performed on two different models, one with modeled ICL3 and another with two open ends covalently bonded. A "very inactive" conformation, which is characterized by a further movement of intracellular half of TM6 towards receptor core, and a close packing of ICL3 underneath the membrane, was observed. Concurrently, the ligand-binding site expanded with Ser207-Asp113 distance, increasing to 18 Å from 11 Å. To investigate the allosteric coupling, bond restraints were applied to critical residues at ligand-binding site, instigating some conformational changes in distant ICL3. Restraining this region to an open state facilitated ICL3 closure, whereas a relatively constrained binding site hindered ICL3 packing. However, the reverse operation, i.e. opening of the packed ICL3, could not be realized by restraining the binding site region to a closed state. Essential dynamics analysis indicated a strong coupling between extra- and intracellular parts of the receptor, implicating functional relevance for allosteric regulation.

P-436

Functional and structural characterization of the GluK2/GluK5 heterotetramer gating mechanismT. Paramo¹, P. M. G. E. Brown², M. R. P. Aurousseau², M. Musgaard¹, D. Bowie², P. C. Biggin¹

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The most widely expressed kainate receptor proteins are GluK2 and GluK5, and in co-expressing cells they are known to preferentially assemble together with a 2:2 stoichiometry, probably due to the high affinity of their amino-terminal domains. Although *in vivo* the GluK2/GluK5 heterotetramer is abundant, to date the most extensively studied kainate receptor construct has been the GluK2 homomer. In order to gain insights into this more physiologically relevant receptor, we have combined *in-silico* structural models and molecular dynamics (MD) simulations with functional studies of the GluK2/GluK5 heterotetramer. Several repeats of long-timescale atomistic MD simulations in the presence and absence of modulatory ions were used to assess the viability of each of the two possible combinations of GluK2 and GluK5 proteins. We find that the different conductance pattern observed in the heterotetramer with respect to the homomer might be accounted for by differences in ion-binding affinities, which in turn affect the channel opening rate. Moreover, we also observe that the GluK5 protein is more robust than GluK2 to the absence of external modulating ions. Thus, GluK5 possesses unique features that help to broaden the functional and pharmacological profile of kainate receptors.

Posters

– 11. Computational biophysics –

P-437

Rational design of multi-motor driven nano-carriers

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A rational design for a nano-carrier (NC) is suggested, that will maximize the arrival efficiency from the plasma membrane to the nucleus surrounding [1]. The design is based on grafting the particle surface with flexible polymer spacers (e.g., PEG) that end with motor protein associating molecules, e.g., nuclear localization signal peptides. This leads to collective active transport of the NC on a single microtubule by several dynein motor proteins. It is theoretically shown that the spacer polymer molecular weight can be adjusted to significantly increase the effective particle processivity time. Including the effects of motor mutual stalling and jamming, and the possible enhancement of motor detachment from the microtubule due to polymer stretching, we show that the polymer molecular weight can be further adjusted to enhance the cargo effective run length to a few tens of microns, much larger than the single motor run length (~1mic.) [2]. This should lead to appreciable enhancement of the active transport of the nano-carrier, and consequently drug delivery, to the nucleus, as we further show using an integrated intracellular transport model.

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P-439

Solution structure of the Mitoxantrone-DNA complex: A NMR and molecular modelling study

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With much effort being directed into the development of novel anticancer agents, some of the most successful chemotherapeutics still remain the ‘classical agents’ and their analogues which target cellular DNA directly. Mitoxantrone is a clinically important antitumor agent that intercalates into DNA and poisons topoisomerase II and is used in the treatment of hormone-refractory prostate cancer, breast cancer and acute myeloid leukaemia. Recently a series of crystal structures of the ternary drug-DNA-topoisomerase complexes were reported, with various anticancer agents including Mitox. In this, the drug adopts a previously unseen “threading” structure with side chains lying in both grooves of the DNA, forming H-bond interactions with the topoisomerase. In contrast, we report the fully assigned spectrum of free d(AACGTT)₂ and its 1:1 complex with Mitox, where it intercalates the CpG step, and its side chains lying in the major groove making H-bonds to the O6/N7 of guanine. 1 us unrestrained molecular dynamics were then performed on both drug conformations with x3DNA being utilized in the analysis of the given structures.

P-438

Fluctuations in the membrane potential of biological cells

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Every living cell is continually adjusting its concentrations of membrane-permeable ions in order to avoid a rise in osmotic pressure. This results in fluctuations of the membrane potential around its resting value. While the average membrane potential can be described by the Goldman-Hodgkin-Katz equation [1], fluctuations require special treatment. Recordings are usually made under voltage-clamped conditions, where the voltage fluctuations cannot be recorded or understood. We model the fluctuations with a conductance based model and an ensemble of membrane oscillators [2]. The model is based on experimental data recorded in a free-running voltage patch clamp experiment [3]. Voltages in non-excitable cells were recorded under different extracellular K⁺/Na⁺/Cl[−] concentrations, or with added intracellular Ca²⁺ ions, or ATP, or both. The results of data analyses and the model will be presented and discussed.

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P-440

Molecular Dynamics Simulations to predict therapeutically exploitable lipid binding sites in GPCRs

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Membrane proteins constitute about 25% of all proteins expressed in a given organism. These proteins are crucial for cell communication and permit signal transfer through the cell membrane. For this reason, membrane proteins are crucial targets for pharmaceutical compounds (~50% of currently marketed drugs). Among membrane proteins GPCRs comprise around 60% of targets. Despite the proven success of GPCRs as drug targets many efforts for selective ligands or drug candidates for GPCRs failed. Thus there is a major need to develop new approaches for the discovery of new therapeutic agents for this important class of receptors.

In this project molecular dynamics simulations are applied to investigate potential binding sites for allosteric modulators. For this aim experimentally solved crystal structures of GPCRs with co-crystallised allosteric modulators have been reinserted in their native environment, a biological membrane and subsequently simulated in atomistic and coarse-grained molecular dynamics simulations. These results will be compared to experimental data of mass spectroscopy.

Posters

– 11. Computational biophysics –

P-441

Prediction of the biochemical mechanisms of action and side effects of the anabolic steroidsD. L. Roman^{1,2}, M. Roman^{1,2}, A. Ciorsac³, V. Ostafe^{1,2}, A. Isvoran^{1,2}¹West University of Timisoara, 16 Pestalozzi, 300115 Timisoara, Romania; ²Advanced Environmental Research Laboratories, 4 Oituz, 300086, Timisoara, Romania; ³Politehnica University Timisoara, 2A P-ta Victoriei, Timisoara, Romania

Anabolic and androgen steroids (AASs) are synthetic drugs and dietary supplements derived from testosterone. The drugs can be used under medical prescription for treating diseases resulting from steroid hormone deficiency or from the loss of muscle mass. They are also illegally used by athletes with the aim to better perform in their specific area of sport and by adults to improve the physical appearance. Literature data concerning the side effects of the AASs are often discordant, the molecular targets of the AASs in the human body are not well known and characterized and molecular mechanisms of the AASs actions are also poorly understood. Within this study, we predict computationally the molecular targets, the molecular pathways and the biological effects of the most common drugs and dietary supplements used as AASs. In addition, we predict if AASs inhibit the cytochromes P450 (CYP) that are involved in xenobiotics metabolism (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4) and we assess these possible interactions by molecular docking. To explore the enzymes flexibility in the molecular docking studies, we consider dissimilar crystallographic structures for every CYP when available.

P-443

Biomolecular force field comparison: Protein-lipid interactions at the membrane interfaceA. Sandoval-Perez, K. Pluhackova, R. A. Böckmann
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Molecular dynamics (MD) allows the study of biological processes at high spatial and temporal resolution. Force field parameters have been developed for a wide variety of molecules ranging from inorganic ligands and small organic molecules over proteins and lipids to nucleic acids. Typically, force fields have been parameterized and validated on thermodynamic observables and structural characteristics of individual compounds. The validation and parameterization based on properties of mixed systems such as protein-lipid interactions have been less tested, due to missing experimental data to compare to and to the complexity of the systems. Therefore, the selection and combination of mixed force fields is particularly complicated. We assessed protein-lipid interactions as described in four force fields GROMOS54a7, CHARMM36, Amber14sb/Slipids and Amber14sb/Lipid14. Four observables were compared: the conservation of secondary structure of transmembrane proteins, positioning of transmembrane peptides relative to the lipid bilayer, insertion depth of side chains of unfolded peptides absorbed at the membrane interface, and reproduction of experimental insertion energies of Wimley-White peptides. Significant differences between force fields were observed. Submitted to JCTC*

P-442

Charged ligand-protected Au nanoparticles interacting with model lipid membranesS. Salassi, F. Simonelli, D. Bochicchio, R. Ferrando, G. Rossi
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The design of ligand-protected metal nanoparticles (NPs) with biomedical applications relies on the molecular level understanding of their interaction with cells. Here, we use coarse grained (CG) molecular dynamics to investigate the interaction between charged monolayer protected Au NPs and model zwitterionic lipid (POPC) membranes^{1,2}. We study the molecular mechanisms and the energetics of the NP-membrane interaction. In particular, we quantify the energy barrier for the anchoring of the NP to the membrane, via the translocation of one or more charged ligands through the hydrophobic lipid membrane core. We characterize the membrane structural behavior during the interaction. Also, we critically discuss different possible choices of the CG force field (FF) modeling the NP-membrane complex. We compare the standard Martini³ FF, which does not include long-range electrostatics and does not account for water polarizability, with two versions of the polarizable-water Martini FF^{4,5}. Our conclusions provide guidelines to the choice of the appropriate model for the simulation of charged NP-membrane complexes.

¹Simonelli, JPCLet 6 (2015) 3175²Salassi, JPCC (2017), in press³Marrink, JPC-B, 111 (2007) 7812⁴Yesylevskyy, PLoS Comp. Biol, 6 (2010) e1000810⁵Michalowsky, JCP 5 (2017) 054501

P-444

Simulation of cellular adhesionF. Savić, B. Geil, A. Janshoff
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The adhesion of cells to the extracellular matrix is an important process in biology. To understand the physical processes involved in the onset of cellular adhesion, especially the lateral organization of adhesion molecules into clusters, we perform Monte-Carlo-Simulations based on a harmonic multispring model involving lipid membranes and their physical properties. Local deformation of the membrane in the vicinity of adhesion clusters facilitates cluster growth while a repulsive interaction between clusters arises due to an interplay of membrane bending rigidity and non-specific repulsion. Balance of this interactions governs cluster size and stability in our simulations.

Posters

– 11. Computational biophysics –

P-445

How do oxidised phospholipids affect the properties of a lipid bilayer?

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Oxidative stress continues to be associated with aging and disease. It is known to affect the pathophysiology of lipids in conditions including, but not limited to, Alzheimer's disease, cystic fibrosis, atherosclerosis, cardiovascular disease and cancer. Lipid peroxidation has a wide and varied impact on cells, and little is known about the effects of oxidative stress on the integrity and biophysical properties of the cell plasma membrane. To address this, we performed a series of microsecond-timescale atomistic molecular dynamics simulations of bilayers containing standard and oxidised phospholipids. We studied the following bilayers: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), POPC + cholesterol, POPC + oxidised POPC (1-palmitoyl-2-(9'-oxononanoyl)-*sn*-glycero-3-phosphocholine (PoxnoPC) or 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PazePC)) and POPC + cholesterol + oxidised POPC (PoxnoPC or PazePC). Our results suggest that membrane composition influences the basic biophysical properties of bilayer systems, including bilayer thickness, permeability to water, area per lipid, bilayer phase and ordering. This is fundamental to our understanding of the effect of lipid peroxidation on biological membranes.

P-447

Palmitoylation & Arrestin as Alternatives to Orthosteric Binding Sites for Modulating GPCR Function

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Involvement of G-protein-coupled receptors (GPCRs) in various pathophysiological pathways makes them excellent drug targets, which has been evidenced by the fact that 40% of currently prescribed drugs target this family. GPCRs have a well-conserved orthosteric ligand binding site which makes challenging to target either a specific receptor or a subtype of the receptor. In this respect, identification of allosteric sites –as alternatives to orthosteric ones– is crucial for development of target-specific and so safer therapeutic molecules with fewer side effects. It has been well-established that the function of the receptor can be modulated by means of either i) post-translational modifications or by ii) adjusting the phosphorylation dependence of Arrestin for binding receptor; however, the underlying molecular mechanisms remain elusive. Here, we present two computational studies (1,2), one of which has been also improved by experiment (3) that provide mechanistic insights into possible roles of i) and ii) in modulation of the receptor function.

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P-446

Solvent accessibility and ligand binding in AcNiR, a two domain copper nitrite reductaseK. Sen^{1,2}, M. A. Hough¹, C. W. Yong², T. W. Keal², R. W. Strange¹

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Microbial copper-containing nitrite reductases (CuNiRs) are enzymes that catalyze the reduction of NO₂⁻ to NO, a key denitrification step in the global nitrogen cycle. This process involves a proton-coupled electron transfer reaction comprising two copper sites, the electron donating T1Cu site and the catalytic T2Cu site. Using multiscale computer modelling we intend to understand the proton and electron transfer pathways coupled with NO₂⁻ reduction at the T2Cu site. Starting from crystal structures of two-domain *Achromobacter cycloclastes* CuNiR (AcNiR) and using classical MD simulations we studied the role of active site residue Asp_{CAT} in modulating water and proton accessibility to the T2Cu site. The protonation of Asp_{CAT} promotes switching between its proximal and gate-keeper positions, both observed crystallographically at low temperature, and facilitates solvent accessibility. Using QM studies, we correlated serial crystallography observations of two binding modes of NO₂⁻ at the T2Cu site to its oxidation state. Knowledge obtained from the studies on solvent accessibility along with oxidation state of Cu coupled to NO₂⁻ binding is incorporated in building models for QM/MM simulations to investigate the underlying mechanistic basis for the observed behaviors.

P-448

Allelic-dependence of MHC I stability on peptide termini contacts in MD simulationsO. Serçinoğlu^{1,2}, P. Ozbek Sariuca²

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Intracellular peptides are presented by the Major Histocompatibility Complex Class I (MHC) molecules to T-cell receptors (TCR) located on the surface of cytotoxic T-lymphocyte cells. Identification of peptides which enable the formation of a stable peptide-loaded MHC I complex is of interest for peptide vaccine design. Previous studies aiming to understand the relationship between peptide termini contacts and MHC stability yielded conflicting results. Recently, the importance of the contacts made by the peptide C-terminus for MHC I stability on a murine MHC I allele (H2Kb) was demonstrated using N- or C-termini truncated peptides [1]. Similarly, we conducted MD simulations with peptides truncated at either terminus bound to H2Kb as well as to two human MHC I alleles, HLA-A*02:01 and HLA-B*27:05. While we could reproduce the H2Kb stability profiles from RMSD distributions, the two human MHC alleles indicated a relatively higher importance of the N-terminus contacts of the peptide for MHC I stability.

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Posters

– 11. Computational biophysics –

P-449

Bayesian refinement of protein structures and ensembles against SAXS data using molecular dynamics

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Small-angle X-ray scattering is an increasingly popular technique used to detect protein structures and ensembles in solution. However, the refinement of structures and ensembles against SAXS data is often ambiguous due to the low information content of SAXS data, unknown systematic errors, and unknown scattering contributions from the solvent. We offer a solution to such problems by combining Bayesian inference with molecular dynamics simulations and explicit-solvent SAXS calculations. The Bayesian formulation correctly weights the SAXS data versus prior physical knowledge, it quantifies the precision or ambiguity of fitted structures and ensembles, and it accounts for unknown systematic errors due to poor buffer matching. The method further provides a probabilistic criterion for identifying the number of states required to explain the SAXS data. The method is demonstrated by refining ensembles of a periplasmic binding protein and of the large chaperone heat shock protein 90 (Hsp90).

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P-451

Anionic/cationic gold nanoparticles interacting with lipid membranes: experiments and simulationsF. Simonelli¹, E. Canepa², F. Canepa², R. Rolandi¹, S. Salassi¹, R. Ferrando², G. Rossi¹, A. Relini¹¹Department of Physics, University of Genova, Italy;²Department of Chemistry, University of Genova, Italy

The interaction between inorganic nanoparticles (NPs) and lipid membranes results from the complex interplay of electrostatics, hydrophobic interactions, ligand composition, surface ligand organization and, on the membrane side, lipid composition and phase. Here we address, by means of experimental and computational techniques, the interaction between monolayer-protected gold NPs and model lipid membranes. We focus on the influence of the NP coating charge (positive or negative) on the interaction with zwitterionic 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membranes. We synthesized Au NPs with a diameter $4 < d < 8$ nm and passivated them with both hydrophobic and charge carrying (negative, $-\text{COO}^-$ terminal group, or positive, $-\text{N}^+(\text{CH}_3)_3$ terminal group) alkanethiols. We quantified the NP-induced leakage from POPC liposomes loaded with calcein, and observed larger calcein release from the liposomes loaded with cationic NPs. Our experimental results are critically discussed in light of the results of biased and unbiased molecular dynamics simulations, describing the structural and thermodynamic features of one possible non-disruptive NP-membrane interaction mechanism^{1,2}. Refs:¹Simonelli JPCLet 6 (2015) 3175; ²Salassi JPCC (2017), in press.

P-450

Molecular dynamics simulations of streptavidin mutant-biotin analog systems

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The biotin-streptavidin (SA) system is known to have a strongest noncovalent biological interaction, and has been used for medical applications such as pre-targeting system. So far, many trials of the system for the clinical application have not been successful due to mainly two problems, the immunogenicity of a bacterium-derived SA and the endogenous biotin species. Recently, our experimental group has developed a pre-targeting system, which overcomes such the two problems. In the system, the SA is modified to decrease the immunogenicity, and the SA mutant binds to an artificial biotin analog while abolishing affinity for natural biotin. In this work, we have performed a series of long molecular dynamics simulations for SA or its mutants with/without biotin analog systems to investigate the effect of mutations on the structural change, dynamics and the thermodynamic properties of the system. We also have calculated free energy of the systems using MPCAFEE method to evaluate binding affinity of artificial biotin analogs with SA mutants. We found that the mutations in the biotin-binding pocket affect not only the interaction with surrounding residues and with biotin analog, but also the tetramerization of the SA mutants.

P-452

Proton transfer in G-C base pairs embedded in solvated DNA: Reaction mechanism and free energies

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In this work we analyze the reaction mechanism and free energy profile of proton transfer in Guanine-Cytosine pairs embedded in solvated DNA. By means of a QM/MM method [1], which combines Fireball (a local-orbital DFT software) with AMBER (a molecular dynamics software) we perform long simulations of the base-pairs in a realistic biomolecular environment. Both Umbrella Sampling simulations and Steered Molecular Dynamics were performed to investigate the proton transfer reaction in Guanine-Cytosine. From them, we extract detailed information of the pathway of the tautomerization reactions, the charge-transfer processes along them and the electronic structure of the base-pair under approximate physiological conditions. We have computed the QM/MM Free Energy profiles of these reactions using three different methods, WHAM, Umbrella Integration and the Jarzynski identity, and we have found an excellent agreement between all of them. Therefore, our work serves also as a test for these Free Energy methods, their convenience and their efficiency for this particular system.

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Posters

– 11. Computational biophysics –

P-453

Allosteric modulation of aromatase: a novel strategy for the next generation anti-cancer drugsA. Spinello¹, M. Pennati², N. Zaffaroni², A. Magistrato¹¹CNR-IOM-Democritos National Simulation Center c/o SISSA, Trieste, Italy; ²Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

The inhibition of Aromatase (AR) is the most used clinical strategy to fight breast cancer. AR catalyzes the conversion of androgens to estrogens in a three-step reaction. Its mechanism is still object of an intense debate, as it is believed to be different from most CYP450s. Recently, it was shown that letrozole (LTZ), the most potent drug, is active via a non-competitive mechanism. Since the crystal structure of AR/LTZ complex has never been solved and LTZ badly fits in the active site, an allosteric binding was hypothesized. Allosteric pockets of AR were identified by the group of Dr. Magistrato and we are now rationalizing the role of those sites. Surprisingly our study, using QM/MM metadynamics, revealed a novel mechanism in which AR performs its catalytic function similarly to all other CYP450s. One allosteric pocket lies exactly between Asp309 and Arg192, that proved to be crucial for the formation of compound I (CpdI) reactive species. Our MD simulations clearly show that LTZ can bind to this allosteric site hampering the proton shuttle essential for CpdI formation. Furthermore, we studied the relationship between LTZ allosteric binding and protein dynamics. Finally, we are developing a novel generation of AR inhibitors, which will be tested by fluorometric essays.

P-455

Predicting the steady-state rate of mRNA translation in protein biosynthesisJ. Szavits-Nossan¹, L. Ciandrini², M. C. Romano³¹SUPA, School of Physics and Astronomy, University of Edinburgh, Peter Guthrie Tait Road, Edinburgh EH9 3FD, UK; ²DIMNP UMR 5235, Université de Montpellier and CNRS, F-34095, Montpellier, France; ³Department of Physics, Institute for Complex Systems and Mathematical Biology, Meston Building, Old Aberdeen, Aberdeen, UK

Existing evidence such as codon usage bias suggests that protein translation of fast-growing organisms has been optimised for accuracy and better yield. One possibility for optimisation stems from the fact that different codons are translated at different rates. Common measures for optimal codon choice, which ignore the dynamics of this process, have so far had mixed success in predicting protein levels. Recent advances in experimental techniques such as ribosome profiling allow us to study dynamical details of this intricate process and theoretical modelling is required to interpret the new data. In this work we study a kinetic model for translation that takes into account stochastic movement of individual ribosomes along the mRNA molecule. Unlike previous studies of this and similar models that were limited to numerical simulations, we develop a novel mathematical method to find the steady state solution when the translation initiation is a rate-limiting step. This allows us, for the first time, to find an analytic prediction for the translation rate and ribosome density. We use these predictions to address some of the long-standing controversies surrounding the determinants of translation efficiency.

P-454

Computational investigation of binding and dynamics in Tom20-mitochondrial targeting signal complexA. Srivastava¹, O. Miyashita², F. Tama^{1,3}¹Department of Physics, Graduate School of Science, Nagoya University, Japan; ²RIKEN Advanced Institute for Computational Science, Japan; ³Institute of Transformative Biomolecules, Nagoya University, Japan

Majority of mitochondrial proteins are transported into mitochondria through the translocase complex of the outer mitochondrial membrane (TOM). Tom20, a major component of TOM complex, recognizes and binds to presequence of mitochondrial precursor proteins. Earlier experimental studies strongly indicated that this binding occurs as a dynamic equilibrium among multiple binding poses, as opposed to the conventional single binding mode. The crystal structures of this complex obtained using disulfide tethering can be classified into A, Y and M poses based on the binding mode of presequence to Tom20. Here, using simulations and experiments, the dynamics of these complexes and the effect of presequence composition has been studied. Molecular dynamics (MD) and Replica exchange MD (REMD) simulations of Tom20/presequence complex (A & Y) have been performed to sample their conformational space. A & Y adopt slightly different conformations as observed from network visualization of REMD trajectories. In order to explore the effect of crystal environment on the binding modes, crystal MD simulations have been performed. The comparison of MD simulation in solution and the one in the crystal environment will provide further insight into different crystal forms.

P-456

How wettable is the skin surface?A. S. Tascini¹, R. Chen², J. M. Seddon¹, F. Bresme¹¹Department of Chemistry, Imperial College London, UK; ²Department of Chemical Engineering, Imperial College London, UK

For humans to survive, the loss of water from the skin must be carefully regulated, with most of the water retained inside the body and a very small amount designated to hydrate the outer layers. On the skin surface, there is a mixture of sebaceous lipids (the sebum oil - SO) and epidermis lipids that detach from the outermost layer of the skin, the Stratum Corneum (SC). The properties of these lipid molecules are fundamental to ensure the low permeability of the skin and to determine its water permeability. Specifically, SO has been shown to play a key role in skin hydration and waterproofing, in the antimicrobial properties of the skin and in the transport of antioxidants.

Here we present Molecular Dynamics computations of the interactions of models of SO with skin lipid bilayers. We assess the interactions of water with the most abundant component of SO, the triglyceride tri-cis-6-hexadecenoin (TG). Furthermore, we quantify the wettability of SC lipids in the presence of water and TG, by computing the contact angle of a water droplet on the surface of the bilayer. A fundamental understanding of the properties of the skin surface is a key step to characterise the skin performance as a barrier and its permeability in applications concerned with transdermal drug delivery.

Posters

– 11. Computational biophysics –

P-457

FimH - Alleles and allosteryM. Thomas¹, M. L. O'Mara¹, M. Totsika²¹Australian National University, Australia; ²Queensland University of Technology, Australia

The fimbrial adhesive protein, FimH, sits at the tip of the multiprotein fimbrial of *E. coli*. It binds to mannosylated surfaces using a catch-bond mechanism, wherein shear forces across the fimbrial increase the binding affinity. Several residues outside the mannose binding pocket have evolved under positive selection. These naturally occurring mutations, termed “pathoadaptive”, can cause commensal *E. coli* to adopt a pathogenic lifestyle in a new host niche by modifying the function of FimH. Whole genome and more specific FimH typing has revealed over 300 different FimH variants, many of which differ from each other by only a single amino acid residue. These small differences can have a significant impact on the FimH phenotype.

To gain insight into how pathoadaptive mutations affect FimH function, the underlying mechanism of allostery in FimH must first be understood. There is still much debate as to how the shear force is transduced to increased binding affinity; whether it be one of many static structure rearrangements, or an alteration in protein dynamics. In this study, we perform extensive molecular dynamics simulations on several FimH alleles to determine: (1) the allosteric mechanism of shear force transduction, and (2) how different alleles modulate this mechanism.

P-459

A computational study of the effect of glycerol on the structure of DPPC monolayers and bilayersJ. L. Trick¹, W. Terakosolphan², B. Forbes², R. D. Harvey³, C. D. Lorenz¹

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Lung Surfactant (LS), a monolayer coating the alveolar surface of the lung undergoes changes during the breathing cycle (1). Glycerol, a known cryo-protectant is known to induce folding (2) in the LS, stiffening (3) in model LS monolayers, modulate area per lipid (4), and possibly the transition temperature (5) of such systems experimentally. Using molecular dynamics (MD) simulations, we model LS as a pure dipalmitoylphosphatidylcholine (DPPC) monolayers and bilayers, in which the concentration of glycerol and simulation temperature are varied to investigate the molecular basis of such variations in behavior. Our Simulations and complementary experimental data suggest a dehydration of the DPPC headgroups, and glycerol interactions with the ester region of the lipid under high concentrations of glycerol. This change could influence the use of glycerol in possible aerosol devices and the permeability of the modulated monolayer.

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P-458

Simulation of lipid membrane damage by nanoparticle-induced localized heating

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Photoporation is a physical approach to permeate cell membranes. Plasmonic nanoparticles (NPs) are attached to the membrane, which is then irradiated by nanosecond laser pulses. Au NPs, which have strong surface plasmon resonance and can efficiently convert light into heat, lead to pore formation in the membrane by localized hyperthermia. Pores then allow for the delivery of, e.g., genetic material to the cell interior, or for the release of the drug content out of a delivery-liposome.

Here, we aimed at modeling, via atomistic Molecular Dynamics simulations, the kinetics of membrane heating and damaging induced by the selective NP irradiation.

We developed a protocol for the simulation of the NP mediated heating in presence of a constant energy flux provided by the laser source, by using reference experimental parameters.

We obtained membrane deformations compatible with experiments. The water permeation kinetics has also been analyzed as a function of the membrane temperature during the heating process.

We observed a non linear dependence between permeability and temperature. Moreover, water permeability is increased at the interface with the nanoparticle.

P-460

Gas diffusion in an O₂-tolerant membrane-bound [NiFe] hydrogenaseT. Utesch¹, J. Kalms², A. Schmidt², S. Frielingsdorf¹, G. Gotthard³, D. von Stetten³, P. van der Linden³, A. Royant^{3,4}, M. A. Mrogiński¹, P. Carpentier^{3,4}, O. Lenz¹, P. Scheerer²

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[NiFe] hydrogenases catalyse the reversible heterolytic cleavage of H₂ into protons and electrons. In most hydrogenases this enzymatic reaction is inhibited by low traces of O₂. The membrane-bound hydrogenase, however, overcomes this problem by reducing O₂ with the help of its unique proximal FeS cluster. In order to understand the function and dynamics of the enzyme at a molecular level and consequently increase its efficiency by bioengineering a detailed description of gas diffusion pathways is of high interest.

Here, the diffusion pathways of H₂ and O₂ gas molecules are investigated by classical molecular dynamics simulations in combination with the novel soak-and-freeze method and X-ray crystallography. We demonstrated that highly occupied O₂ positions detected in the experiments perfectly agree with the observations of gas densities in the computational approach. Additionally, the simulations showed that O₂ and H₂ mainly followed a well-defined tunnelling network consisting of two main channels. The observed ‘hopping’ kinetics in this tunnel system was analysed by a simple transition state model thus offering detailed insights into the gas diffusion kinetics.

Posters

– 11. Computational biophysics –

P-461

Ion selectivity in VDAC studied by molecular simulations: role of salt and lipid environment

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The voltage-dependent anion channel (VDAC) serves as the major pore for metabolites and electrolytes in the outer mitochondrial membrane. To refine our understanding of ion permeation through this channel we performed a study of permeation of several small inorganic ions using Brownian dynamics (BD, all-atom molecular dynamics (MD) and coarse-grained molecular dynamics (CG-MD). The salt concentration dependence of VDAC selectivity as well as the role of different lipids were examined. All three computational methods show the VDAC pore becomes less anion selective upon increasing salt concentration although the dependence is less marked with CG-MD. Our simulations also point to the role the nature of the lipid head groups may have on the selectivity of the channel.

P-462

Mechanism and energetics of substrate transport by multi-drug RND antiporters unveiled in silico

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Multi-drug efflux pumps such as AcrAB-TolC confer resistance to many structurally unrelated antibiotics in *Escherichia coli* and other Gram-negative bacteria. Understanding the molecular functioning of these pumps is fundamental for basic research and for drug design purposes. A functional rotation mechanism of substrate transport was proposed for AcrB, but no study has so far provided clear-cut evidence thereof. Moreover, molecular determinants and the energetics of the mechanism have yet to be assessed. Here, we propose a novel computational protocol employing multiple-bias simulations to thoroughly mimic functional rotation, assess its feasibility and evaluate for the first time its energetics. The calculated free energy profile is smooth and compatible with available data. Interestingly, a “thermodynamic inversion” occurs along the functional rotation in the binding free energies of the substrate between the recognition site and the putative exit gate from AcrB. Our protocol can be applied to compounds for which no experimental information is available, as well as to transporters homologous to AcrB.

P-463

Human embryonic stem cell colony formation: statistical analysis and agent-based modelling

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Numerous biological approaches are available to characterise the mechanisms which govern the formation of human embryonic stem cell (hESC) colonies. To understand how the kinematics of single and pairs of hESCs impact colony formation, we study their mobility characteristics using time-lapse imaging. We perform a detailed statistical analysis of their speed, survival, directionality, distance travelled and diffusivity and confirm that single and pairs of cells migrate as a diffusive random walk for at least 7 hours of evolution. We show that the presence of Cell Tracer significantly reduces hESC mobility. Our results open the path to employ the theoretical framework of the diffusive random walk for the prognostic modelling and optimisation of the growth of hESC colonies. To model non-trivial cell-cell interactions we have extended the random walk model to incorporate an effective Lennard-Jones (molecular-like) potential between the cells, which is now being extended towards colony formation to include the effects of cell division, cell cycle, death and differentiation into specialised cells. We aim to develop a stochastic spatiotemporal model (using agent-based modelling combined with stochastic equations) capable of modelling hESCs from single cells up to colonies of thousands.

P-464

Extension of replica-permutation molecular dynamics method to NPT ensemble and its application

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Molecular dynamics simulation is a powerful tool to study biomolecules. However, sometimes their configurations get trapped in local minimum of free energy landscape and it prevents us from efficient simulations. To overcome this problem, replica-exchange method and replica-permutation method have been proposed. Replica-permutation method is a better alternative of replica-exchange method. However, volume cannot be changed in previous replica-permutation method. In this study, we developed isothermal-isobaric replica-permutation molecular dynamics method.

To compare its sampling efficiency with isothermal-isobaric replica-exchange method, we calculated tunneling events and transition probability of parameter labels. These results show that replica-permutation method is more efficient than replica-exchange method.

Furthermore, we applied this method to a β -hairpin mini protein, chignolin. In this simulation, we observed folding-unfolding events many times. We will discuss the detail behavior of chignolin depending on temperature and pressure and show new insight about chignolin in my poster presentation.

Posters

– 11. Computational biophysics –

P-465

Characterisation of biofilm surface height variations using agent-based models

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Bacterial biofilms are implicated in many chronic infections because they can resist the body's immune system and can tolerate high concentrations of antibiotics. One factor which influences a biofilm's function in this respect is its spatial structure through, for example, affecting the ability of nutrients or antibiotics to penetrate the biofilm. This study uses an established agent-based biofilm modelling software known as iDynoMiCs to characterise biofilm surface height variations in different growth regimes for *Pseudomonas aeruginosa*-like biofilms. Within the regime characterised by the presence of biofilm "fingers", we identify two qualitatively different growth phases that are distinguished by their relationship between the roughness and the active layer depth. The first phase, corresponds to high substrate concentrations and low maximum growth rate. Here, the active layer extends across the entire biofilm and fluctuations in the active layer depth drive the increase in roughness. The second phase, corresponds to lower nutrient concentrations and lower maximum growth rates. Here, the active layer is discontinuous and the troughs of the biofilm fingers become stationary, or 'pinned'.

P-467

The cooperation of transcription factor binding and histone modification in two cell lines

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The cooperation among transcription factors (TFs) and histone modifications (HMs) is important dynamics process for the precise control of gene expression in normal and disease state. We extracted signal peaks data of 55 TFs binding and 11 HMs and the RNA-seq data in human GM12878 and K562 cell lines. The distribution difference of 55 TFs binding and 11 HMs were analyzed in the upstream and downstream regulatory region of transcription start sites (TSS). In order to the reveal the possible cooperation between TF and TF or between TF and HM, the degree of TF-TF or TF-HM overlapping are defined by introducing a set of simple indexes in TSS region and whole genome. Some interesting TF and HM cooperation pattern are found. Furthermore, by considering the downstream gene expression levels from RNA-seq data, we built two kinds of gene sets with high and low expression level. Based on the TF or HM association strength, two kinds of gene were well identified by using the SVM models with different parameters. Our results indicated that prediction power depends on the selection of TFs or HMs and the highest prediction accuracy is 93%.

Keywords: transcription factor; histone modification; cooperation

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P-466

Anion- π interactions in flavoproteins involve a substantial charge-transfer componentY. P. Yurenko^{1,2}, S. Bazzi³, R. Marek¹, J. Kozelka^{2,4}

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Anion- π interactions occur in flavoproteins and regulate the redox potential of the flavin cofactor. They are commonly considered to be of electrostatic nature. Here we question this view and show that anion-flavin interactions can have a significant covalent component.

We examined the nature of anion-flavin interactions using two energy decomposition schemes, the Ziegler-Rauk EDA method and the Natural Energy Decomposition Analysis. We used 7,8,10-trimethylisoalloxazine as a model for the flavin cofactor occurring in the prosthetic groups FMN and FAD, and analyzed its gas-phase interaction energy with Cl^- , N_3^- , and SO_4^{2-} . These anions were recently shown to interact with the π -face of flavin in crystallized flavoenzymes. Our analysis indicates that anion-flavin interactions can have a substantial charge-transfer component, arising from favorable overlap between lone-pairs of electrons of the anion with empty π^* orbitals of the flavin aromatic system. Our conclusion is in line with reported observations of absorption bands, originating from charge transfer between oxidized flavin and proximate cysteine thiolate groups.

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P-468

Modelling cellular blood flow in microcirculatory bifurcationsQ. Zhou¹, M. O. Bernabeu², T. Krüger¹

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Red blood cells (RBCs) are a fundamental blood constituent responsible for transporting oxygen to and carbon dioxide away from tissues through the microcirculation (Popel & Johnson, 2005), which consists of extensive small vessels and contains various junctions and bifurcations. Because the partitioning of RBCs at a bifurcation is often uneven (Secomb, 2017), either physiological or pathological phenomena can arise as a consequence in downstream vessels, e.g. plasma skimming, vessel regression, capillary branching, etc. However, the cell behaviour itself as blood flow diverges into daughter branches is still not entirely clear.

To explore RBC dynamics in the microcirculatory system and shed light on the underlying biophysical implications, we employ an immersed boundary-lattice Boltzmann model to simulate cellular blood flow in a microfluidic network incorporating hierarchical bifurcations. The model is based on a highly-parallelised open source LB flow solver, HemeLB (Mazzeo & Coveney, 2008), with a newly added element of discrete RBCs. We aim to unravel the mechanism governing cell distribution at a bifurcation and cell migration in post-bifurcation vessels, and furthermore, relate the findings to the development of cell free layer as well as the pattern of wall shear stress.

Posters

– 12. Protein misfolding –

P-469 (O-73)

Interaction between amyloid oligomers and plasma membrane. A single cell force spectroscopy studyC. Canale¹, R. Oropesa-Nunez¹, S. Dante¹, C. Cecchi², M. Stefani², F. Chiti², A. Diaspro¹¹Nanophysics Department – Istituto Italiano di Tecnologia, Genova, Italy; ²Department of Biochemical Sciences – University of Florence, Florence, Italy

Cytotoxicity in amyloid diseases is mediated by small oligomeric species and, in particular, by their interaction with the plasma membrane. The level of toxicity depends from both the physicochemical properties of the protein aggregates and the composition and organization of the cell membrane.

We employed the small peptide HypF-N as a model of amyloidogenic peptide.

Considering these well demonstrated points:

1. HypF-N aggregates in different conditions, producing oligomers with different toxicity
2. cytotoxicity is always triggered by GM1 content in the cell membrane

We studied the interaction of the cell membrane with toxic and nontoxic protein misfolded oligomers by using AFM-based single cell force spectroscopy (SCFS). We quantified the affinity of the different oligomeric species for the lipid and protein fraction of the cell membrane. In particular, we observed, for the first time, that toxic oligomers influence the functionality of a large class of molecules involved in cell adhesion. We demonstrated that the ganglioside GM1 play a pivotal role in this mechanism.

P-471 (O-75)

Functional amyloids from the fungal pathogen *Aspergillus fumigatus*A. Pillé¹, J. Lai³, V. Lo³, V. Aïmanianda², A. Beaussart⁴, I. Valsecchi², A. Kwan³, J. Bayry⁵, Y. Dufresnes⁴, J.-P. Latgé², M. Sunde³, I. Guijarro¹

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Hydrophobins are fungal proteins characterised by their amphipatic properties and an idiosyncratic pattern of eight cysteine residues. The soluble form of these proteins spontaneously self-associates at hydrophobic/hydrophilic interfaces to form **amphipatic layers with remarkable physicochemical properties**. The **RodA** hydrophobin of the airborne fungal pathogen *Aspergillus fumigatus* forms a functional **amyloid layer** with rodlet morphology that covers the surface of the spores, rendering the latter hydrophobic and thus facilitating their dispersal. In addition, the **rodlet coat masks the spores**, which are the infectious morphotype, **from the immune system**. We have solved the solution structure of RodA, studied its self-assembly *in vitro*, performed a mutational analysis to highlight the regions involved in the formation of the amyloid core of the rodlets and on their lateral association to form layers, correlated the kinetics of rodlet formation *in vitro* with their rate of appearance on the spores and analysed the relationship between the structure of RodA and its immunological properties.

P-470 (O-74)

DNA PAINTing amyloid aggregatesM. H. Horrocks¹, D. Whiten¹, Y. Zuo¹, L. Calo¹, M. G. Spillantini¹, S. Gandhi², D. Klenerman¹
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The aberrant misfolding and aggregation of soluble proteins into amyloid fibrils characterizes many neurodegenerative disorders, including Parkinson's and Alzheimer's disease. The ability to study such processes has remained difficult due to the heterogeneity and low abundance of the aggregates along the fibril-formation pathway. Many such species are smaller than the diffraction limit of light (~250 nm), and so imaging them in high enough resolution with optical microscopy has been limited. We report here a method, termed ADPAINT (aptamer DNA PAINT), for the characterization of amyloid species at the nanometer scale. Using a combination of DNA PAINT and an amyloid specific aptamer, we demonstrate that this technique is able to detect a whole range of aggregates species along the aggregation pathway of alpha-synuclein, allowing for the earliest formed oligomers as small as 50 nm to be imaged in high detail, both within cells and in the test tube.

P-472

Unifying view on the impact of GM1 in the oligomerisation of membrane bound Aβ monomersM. Amaro¹, R. Šachl¹, R. Vácha², M. Hof¹

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Oligomers of the β-amyloid (Aβ) peptide have been implicated in Alzheimer disease (AD). Neuronal membranes may mediate Aβ's oligomerisation in brain. Using the single-molecule sensitivity of fluorescence, we address oligomerisation of Aβ monomers on lipid bilayers containing components of the neuronal plasma membrane. We find sphingomyelin to be a trigger of Aβ's oligomerisation and physiological levels of GM₁, organised in nanodomains, not to catalyse oligomerisation (contrary to high GM₁ levels). Moreover, GM₁ prevents oligomerisation, counteracting sphingomyelin's effect. This preventive role of GM₁ suggests that reduction of GM₁ in the brain, e.g. due to aging, could decrease protection against oligomerisation and contribute to AD onset. We suggest the scenarios "GM₁ as catalyst or as inhibitor of Aβ's oligomerisation" are not mutually exclusive but complementary, and might depend on GM₁ organisation in membranes.^{1,2} Of novelty, GM₁ nanoheterogeneities (<26nm, unresolvable by super-resolution microscopy) were uncovered by combining Monte Carlo Simulations, FLIM-FRET³ and FCS techniques using our fluorescent GM₁ analogues⁴.

1-Amaro, et al. *Ang.Chem.* **2016** 55. 2-Cebecauer, Hof, Amaro. *Bioph. J.* **2017** in press. 3-Amaro, et al. *Bioph. J.* **2014** 107. 4-Šachl, et al. *BBA-Mol.C. Res.* **2015** 1853

Posters

– 12. Protein misfolding –

P-473

Curcumin derivatives as a potential theranostic agents for Alzheimer's diseaseZ. Bednarikova¹, G. Orteca², E. Ferrari², M. Asti², M. C. Menziani², Z. Gazova¹¹Dept. of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Slovakia; ²Department of Chemical and Geological Sciences, University of Modena, Italy

Alzheimer's disease (AD), the most common form of dementia, is characterized by the accumulation of aggregated proteins in a form of amyloid plaques and neurofibrillary tangles. We have tested the binding and destroying activities of 12 curcumin derivatives with different scaffold modifications on A β ₄₀ fibrils *in vitro* using spectroscopic and microscopic techniques. The results showed that curcuminoids are able to interfere with A β ₄₀ fibrils depending on their structure. The derivatives modified by adding the methoxy and acetate groups to curcumin scaffold possessed the highest destroying activity (DC₅₀ values in low μ M range). The least effective were derivatives with no substituent. *In silico* calculation confirmed the *in vitro* results. Our results pointed out the anti-amyloid properties and also suggest high affinity of selected curcumin derivatives for A β ₄₀ fibrils. Therefore, we believe that radiolabeled curcuminoids could be potential diagnostic agents in means of PET imaging as well as potential therapeutics for AD.

This work was supported by the research grants from Slovak Grant Agency VEGA 2/0145/17 and VEGA 2/0176/14, the FONDAZIONE DI VIGNOLA (project PHYTORAD) and UNIMORE FAR2015 grant.

P-475

The structural response of HSA to oxidation: a biological buffer to local formation of hypochloriteA. del Giudice¹, C. Dicko², L. Galantini¹, N. V. Pavel¹¹Department of Chemistry, Sapienza University of Rome, Italy; ²Department of Chemistry, Division for Pure and Applied Biochemistry, Lund University, Sweden

The most abundant plasma protein, human serum albumin (HSA), plays a key part in the body's antioxidant defense against reactive species. This study was aimed at correlating oxidant-induced chemical and structural effects on HSA.

Despite the chemical modification induced by the oxidant hypochlorite, the native shape is preserved up to oxidant/HSA molar ratio <80, above which a structural transition occurs in the critical range 80–120. This conformational variation involves the drifting of one of the end-domains from the rest of the protein and corresponds to the loss of one-third of the α -helix and a net increase of the protein negative charge. The transition is highly reproducible suggesting that it represents a well-defined structural response typical of this multidomain protein.

The ability to tolerate high levels of chemical modification in a folded or only partially unfolded state, as well as the stability to aggregation, provides albumin with optimal features as a biological buffer for the local formation of oxidants.

P-474

Characterization of monomers of amyloidogenesis by computer simulations: benchmarking methodsS. Bhattacharya¹, J. L. Dominguez², L. Xu¹, D. Thompson¹¹Bernal Institute, University of Limerick, Limerick, Ireland;²International University of Catalonia, Barcelona, Spain

Conformational switches between monomers in an ensemble of intrinsically disordered proteins (IPDs) such as amyloid-beta, alpha-synuclein, prion and tau might potentially act as the most fundamental trigger for the pathogenesis of neurodegenerative disorders like Alzheimer's, Parkinson's, Prions diseases etc. This necessitates a thorough exploration of the IDP conformational landscape through computer simulations. However, the methods (protein force fields and water models) that are used to simulate IDPs in general have been inaccurate, when compared to experiments. We have systematically explored and benchmarked eight semi-empirically chosen combinations of protein and water models using microsecond scale *de novo*, all-atomistic molecular dynamics simulations to accurately investigate the conformational space of the four 'amyloidogenic' IDPs. The models were validated against experimental NMR, small-angle scattering (SAS) data and other available experimental observables. Our results indicate that the methods which give larger weight to the protein-water interactions show a better match with experimental data, and a substantial improvement over the commonly used protein/water models. This provides a potentially predictive model for properties of new and yet poorly characterised IDPs.

P-476

Ultrasensitive measurement of Ca²⁺ influx into lipid vesicles induced by protein aggregatesP. Flagmeier¹, S. De¹, D. C. Wirthensohn¹, S. F. Lee¹, C. Vincke², S. Muyldermans², T. P. Knowles¹, S. Gandhi³, C. M. Dobson¹, D. Klenerman¹¹Department of Chemistry, University of Cambridge, UK;²Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Belgium; ³Department of Molecular Neuroscience, Institute of Neurology, University College London, UK

In order to quantify and characterize the potentially toxic protein aggregates associated with neurodegenerative diseases, we developed a high throughput assay based on measuring the extent of aggregate induced Ca²⁺ entry into individual lipid vesicles. This approach was implemented by tethering vesicles containing a Ca²⁺ sensitive fluorescent dye to a passivated surface and measuring changes in the fluorescence as a result of membrane permeation using total internal reflection microscopy. Picomolar concentrations of A β ₄₂ oligomers could be observed to induce Ca²⁺ influx, which could be inhibited by the addition of a naturally occurring chaperone and a nanobody designed to bind to A β peptide. The assay can be used to study aggregates from other proteins, such as α -synuclein, and to probe the effects of complex biofluids, such as cerebrospinal fluid, and thus has wide applicability. Potentially, our method enables the quantitative measurement of any biochemical process which involves membrane permeabilization and subsequent Ca²⁺ influx.

Posters

– 12. Protein misfolding –

P-477

Effect of amyloid oligomerization on α -synuclein curvature-membrane sensitivityJ. I. Gallea¹, C. Mas¹, E. E. Ambroggio¹, N. James², D. Jameson², M. S. Celej¹¹Dto. Química Biológica-CIQUIBIC, Fac. Ciencias Químicas, Universidad Nacional de Córdoba, Argentina; ²Dept. Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa, USA

The protein α -synuclein (AS) is as a critical regulator of synaptic vesicle dynamics in dopaminergic neurons. The amyloid aggregation of AS is pathognomonic of Parkinson's disease, a movement disorder associated with axon degeneration of dopaminergic nigral neurons. Prefibrillar oligomeric species are pointed as highly neurotoxic since they can both alter the physiological function of AS and act as active pathogenic species. AS has a greater affinity for highly curved vesicles, such as that of synaptic vesicles. Here, we aimed at determining the loss-of-function that might be associated to the conversion of AS from its monomeric functional state to its pathological oligomeric form by evaluating the impact of AS oligomerization on its membrane-curvature sensitivity. We used Fluorescence Correlation Spectroscopy to obtain quantitative information on the interactions between monomeric and oligomeric AS and vesicles varying in sizes at physiological ionic strength. Our results show that oligomeric AS preserves its curvature-membrane sensitivity, exhibiting a higher affinity for smaller vesicles, but that it binds to vesicles with a slightly lower affinity than the monomeric protein. Our findings provide insight into how amyloid oligomerization can modulate AS physiopathology.

P-479

Multifunctional tacrine-coumarin hybrid molecules as a potential therapeutics of Alzheimer's diseaseZ. Gazova¹, Z. Bednarikova¹, S. Hamulakova², M. Valko³, K. Kuca⁴¹IEP Slovak Academy of Sciences, Slovakia; ²Institute of Chemistry, Faculty of Science, Safarik University, Slovakia; ³Faculty of Chemical and Food technology STU, Slovakia; ⁴Biomedical Research Centre, University Hospital Hradec Kralove, Czech Republic

Alzheimer's disease is a multifactorial disease characterized mainly by amyloid β deposits, cholinergic deficit and extensive metal (Cu, Fe)-induced oxidative stress. We have investigated the anti-amyloid, antioxidant, copper-chelating properties and anti-cholinesterases activities of new multifunctional tacrine-7-hydroxycoumarin hybrids 5a-5g. It was found that hybrids are able to interfere with $A\beta_{40}$ aggregation in a concentration-dependent manner. Compound 5c containing an eight-carbon linker possessed the highest inhibitory activity ($A_{inh} = 81.5\%$). Compounds 5e-g have the most efficient scavenging effect against ABTS+. The interaction of the hybrids with copper ions slightly enhanced their radical scavenging activity. Most of the studied compounds inhibited hAChE at nanomolar concentrations; the most effective was compound 5g with IC_{50} about 38 nM. These results suggest that multifunctional agents, containing acetylcholinesterase inhibitory segment and also antioxidant moiety are promising strategy in the treatment of Alzheimer's disease. Supported by grants VEGA 2/0145/17, 1/0765/14, 1/0041/15, 2/0176/14.

P-478

An effect of small compounds from traditional Chinese herbs on $A\beta_{42}$ aggregation in ADM. Gancar¹, Z. Bednarikova¹, R. Wang², L. Ma², D. Fedunova¹, Y. Tang², B.-B. Zeng², Z. Gazova¹¹Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia; ²East China University of Science and Technology, Shanghai, China

The aggregation of the 42-residue form of the amyloid- β peptide is a central event in Alzheimer's disease (AD). By using various biophysical methods we studied effect of a pool of compounds extracted from traditional herbs from eastern Asia on $A\beta_{42}$ aggregation. Our point was to prove, if any of these compounds have substantial inhibitory/reverse impact on $A\beta_{42}$ aggregation and thus they could be used as a platform for future therapeutics of AD. The anti-amyloid activities (IC_{50} and DC_{50} values) were quantified using Thioflavin T assay and subsequently the obtained results were confirmed by atomic force microscopy. Remarkable results were observed in case of several samples, the most significant inhibitory/reverse activities were detected for two different compounds extracted from the root of *Salvia miltiorrhiza*. Our results show that this strategy represents an early insight to compound's effect on aggregation process and thus a good approach to systematically identify small molecules that inhibit or in other ways affect amyloid aggregation.

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P-480

Adsorption and aggregation of hIAPP at different self-assembled monolayers

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Misfolding and aggregation of the peptide hormone human islet amyloid polypeptide (hIAPP) plays an important role in the development of type2 diabetes mellitus (T2DM). Understanding the interaction of hIAPP with membranes and especially the influence of the membrane properties therefore represents an important goal. In this work, we investigate the adsorption and aggregation of hIAPP at self-assembled monolayers (SAMs) on gold surfaces that present different functional groups, i.e., NH_2 , OH , $COOH$, and CH_3 . The dynamics of hIAPP adsorption on these surfaces is studied in situ using quartz crystal microbalance with dissipation, while the morphology of the aggregates is analyzed by ex-situ atomic force microscopy. We observe a strong influence of the functional group on hIAPP adsorption and fibril formation. In particular, the negatively charged COO^- terminated SAM is found to promote fibrillation, in agreement with previous observations on other negatively charged surfaces and membranes. In order to obtain a deeper understanding of the relevant mechanisms that control the surface-catalyzed aggregation of hIAPP, these results are compared to additional experiments with rat IAPP that is not as prone to aggregation as the human variant.

Posters

– 12. Protein misfolding –

P-481

Stability and structural change in the pathological polymerisation of α 1-antitrypsin

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The plasma protease inhibitor α 1-antitrypsin is marginally stable; mutations can result in conformational change producing a hyperstable and inactive polymer. In individuals homozygous for the Z (E342K) mutation, polymers deposit in the liver, predisposing to liver disease and causing a protease-antiprotease imbalance in the lung which can lead to early-onset emphysema. To explore the structural and energetic aspects of the pathway of polymer formation, we have generated a molecular toolkit of conformation-specific monoclonal antibodies (mAbs). We have identified a mAb that can induce pathological behaviour in the wild-type protein, by promoting formation of an intermediate with structural characteristics of the polymer, and conversely a mAb that prevents polymerisation in a cell model of disease. In localising the epitopes of these, and other, antibodies, we have identified a region of α 1-antitrypsin not previously associated with regulating conformational change. We have then characterised changes that occur during polymerisation using site-specific labelling in conjunction with the electron paramagnetic resonance (EPR) and Förster resonance energy transfer (FRET) techniques.

P-483

Self and Cross seeding of A β M(1-40) wild type vs charge mutant peptides

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Amyloid fibrils are formed by ordered aggregation of polypeptides and proteins. Many amyloidogenic proteins are associated with neurodegenerative diseases including Alzheimer's disease. The mechanism of formation of fibrils varies between different amyloid peptides that exist *in vivo*, still seems to contain a small set of microscopic steps: primary nucleation and elongation and possibly also fragmentation or surface catalyzed secondary nucleation. To understand the sequence determinants of the mechanistic details, a set of eight charge mutants of A β M(1-40) that differ in aggregation kinetics were studied. Self and cross seeding of mutant and wild type peptides were performed using ThT as reporter of the aggregation kinetics. We identify that mutation in the N-terminal tail and core region produce different behaviors. We also identify a set of different seeding effects:

1. The identity of the seed determines the effect
2. The identity of the monomer determines the effect
3. The identity of both is critical

P-482

Protein dynamics and conformational disease: characterisation of alpha-1-antitrypsin by NMR

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Alpha-1-antitrypsin (α 1AT) is a 52 kDa serine protease inhibitor found at high concentrations in human plasma. The Z mutation (E342K) causes the formation of polymers that are retained in hepatocytes. Accumulation of polymers leads to liver cirrhosis and the reduced anti-protease activity in the lung predisposes individuals to early onset emphysema. The rational design of therapeutics for the treatment of α 1AT deficiency requires an understanding of the polymerisation pathway. Polymerisation is thought to progress from a native species of an unknown conformation. A recent crystal structure of this Z mutant revealed few differences to the WT variant, suggesting that changes in aggregation behaviour are due to differences in structural dynamics between variants. We have used solution state Nuclear Magnetic Resonance (NMR) and biochemical approaches to characterise the structure, dynamics and polymerisation of α 1AT. In particular, NMR observations of [²H, ¹³C-ILV] labelled α 1AT have allowed us to study dynamics in α 1AT across a range of time scales. Additionally, we show that it is possible to acquire high quality ¹H-¹³C NMR correlation spectra of patient-derived WT and Z- α 1AT at natural isotopic abundance. These data probe the structural and dynamic consequences of the Z mutation.

P-484

Inhibition of lysozyme amyloid fibrillization by phospholipids. Focus on long-chain DMPC

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Recent studies suggest significant influence of phospholipid molecules on formation of amyloid aggregates. We have studied effect of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) on amyloid aggregation of HEW lysozyme *in vitro*. The interference of phospholipids with lysozyme amyloid fibrillization was investigated using spectroscopic techniques, atomic force microscopy and image analysis. Both phospholipids are able dose-dependently inhibit formation of lysozyme fibrils. The length of the phospholipid tails affects the extent of inhibitory activity; long chain DMPC inhibits fibrillization more efficiently. We suggest that inhibitory activity is due to the interaction of phospholipids with lysozyme leading to the blocking of the contacts important for formation of the cross- β structure within the core of the fibrils. Interestingly, DMPC has no destroying effect on the lysozyme amyloid fibrils. The obtained results suggest that DMPC and DHPC represent agents able to modulate lysozyme amyloid aggregation.

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Posters

– 12. Protein misfolding –

P-485

Measuring the prion-like character of tau by TIRF microscopy

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Increasing evidence suggests that neurodegenerative diseases such as Alzheimer's disease share common molecular features with prion disorders. Prions are protein aggregates capable of self-replication, allowing their spread through the brain with fatal outcome. In this study we assessed the ability of the Alzheimer's protein tau to self-replicate in a prion-like manner. Using TIRF microscopy we followed the evolution of single tau aggregates over two months. This revealed a short fibril elongation phase followed by a phase during which existing fibrils undergo slow spontaneous fragmentation. This process increases the concentration of toxic and seeding competent tau species, providing a possible mechanism for the prion-like replication of tau. To corroborate this hypothesis, we determined the rate constants for the elongation and fragmentation of tau and performed simulations of tau aggregate propagation. Importantly, these are able to recapitulate the observed timescales of tau spreading in mouse and human brains and offer an explanation for the slow initiation of AD followed by a rapid decline. This is the first time the key molecular processes of tau replication have been identified and employed to provide a quantitative model for the propagation of tau through the brain.

P-487

Amyloid β -peptide aggregation and interaction with yeast cells membranes

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Protein misfolding, aggregation and conversion into amyloid fibrils is related to a series of neurodegenerative pathologies as Alzheimer's disease. In particular, interactions of amyloidogenic proteins with cell membranes leading to mutually disruptive structural perturbations are considered key factors in regulating amyloid fibrils formation in cellular environment and related toxicity mechanisms.

Here, we present an experimental study on Amyloid β -peptide aggregation at live *Saccharomyces Cerevisiae* yeast cell membranes by means advanced fluorescence microscopy techniques. Fluctuation methods are used to analyse the aggregation process in real time quantitatively mapping membrane mediate aggregation and oligomers formation. 2-photon microscopy and Fluorescence lifetime imaging microscopy are used to analyse peptide-membrane interaction and its effects on live yeast cells giving complementary information on their biological response.

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Non-perturbative single-molecule imaging of tau aggregates by genetic code expansion

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The aberrant aggregation of tau into intracellular deposits is thought to play a key role in the pathogenesis of Alzheimer's disease and other human tauopathies. Many methods that are used to study protein aggregation *in vitro* and *in vivo* rely on the covalent attachment of a label to the protein of interest. However, amyloid proteins such as tau are highly susceptible to mutations or covalent modifications, necessitating the careful selection of an appropriate labelling strategy to maintain native protein behaviour. Here, genetic code expansion is utilised to introduce a well-tolerated biotin-tag near the N-terminus of a pathological mutant of full length tau. Using a range of single-molecule methods such as sm-FRET spectroscopy and DNA-PAINT, we demonstrate that this biotin-tag can be used to study different aggregates of full length human tau – such as small oligomeric nuclei or mature fibrils – with unprecedented detail.

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Mechanisms of Amyloid- β 42 oligomer formation from kinetic analysis

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Alzheimer's disease is intimately connected with the aggregation of the Amyloid-beta 1-42 (A β 42) peptide into amyloid fibrils. There is increased evidence that low-molecular weight, pre-fibrillar oligomers formed during the early stages of amyloid aggregation, rather than the fibrils themselves, are associated with highest toxicity, yet it has remained challenging to characterize the molecular-level processes through which these species are generated. Here, we present a kinetic analysis of oligomer populations formed during A β 42 aggregation to shed light on their molecular mechanisms of formation. We show that A β 42 oligomers, which are predominantly generated through secondary nucleation on the surfaces of existing fibrils, are structurally distinct from fibril nuclei. Moreover, we find that the majority of A β 42 oligomers do not grow into amyloid fibrils, instead predominantly dissociate back to monomeric form. Our results provide important insights into the physical determinants of the autocatalytic formation of amyloid fibrils, providing fundamentally new targets for interventions to prevent the production of neurotoxic oligomeric species.

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– 12. Protein misfolding –

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Structural characterization of protein aggregates and amyloid fibrils by CD spectroscopyA. Micsonai¹, J. Kun¹, F. Wien², Y.-H. Lee³, Y. Goto³, M. Réfrégiers², J. Kardos¹¹MTA-ELTE NAP B Neuroimmunol. Res. G., Dept. Biochem., Eötvös Loránd Univ., Hungary; ²Synchrotron SOLEIL, Gif-sur-Yvette, France; ³Inst. Prot. Res., Osaka Univ., Japan

Determination of the structure of protein aggregates is a difficult. High resolution methods as X-ray and NMR are problematic because of the large size, inhomogeneity, and often insolubility of the aggregates. Our aim was to develop further our new method for protein secondary structure (SS) determination by circular dichroism (CD) spectroscopy to characterize the relationship between structure and (patho)physiological function of amyloid aggregates. Previously available algorithms for CD analysis often failed to provide reliable estimations on β -sheet-rich structures, such as that of amyloid fibrils. We recorded the CD and SRCD spectra of various aggregated forms of different amyloidogenic proteins, such as amyloid- β , alpha-synuclein, β 2-microglobulin, polyglutamin, and insulin, associated with different degenerative diseases. Aggregate morphology was investigated by EM. Using our recently published algorithm (<http://bestsel.elte.hu>) we were able to distinguish structural variants regarding their SS composition including the orientation and twist of β -sheets. This fast and inexpensive technique can provide quantitative and detailed secondary structure information which can be related to aggregate morphology and pathological effect.

P-491

The effect of amyloid β peptide(1–40) on the lipid membrane: a neutron scattering studyC. Ricci¹, P. Falus², F. Librizzi³, M. Maccarini², M. R. Mangione³, O. Moran⁴, M. G. Ortore¹, R. Schweis², S. Vilasi³, R. Carrotta³¹DiSVA, Università Politecnica delle Marche, Ancona, Italy; ²Institut Laue-Langevin, Grenoble, France; ³CNR, Institute of Biophysics, Palermo, Italy; ⁴CNR, Institute of Biophysics, Genova, Italy

Alzheimer's disease (AD) represents a common cause of dementia in the elderly population. Significant evidences suggest that prefibrillar oligomers, constituted of the 39–42 amyloid beta peptide (A β), arising at the beginning of its aggregation process, represent the most toxic species¹. It has been reported that A β can bind the ganglioside GM1, expressed on the extracellular surface, particularly on synaptic membranes. The interaction of GM1 with A β has been previously investigated by x-rays and neutron scattering, using membrane model systems^{2,3}. We present a neutron scattering study on the interaction of large unilamellar liposomes (LUV), as cell membrane models, in presence of both A β monomers and of the early toxic prefibrillar oligomers. Small Angle Neutron Scattering and NSE experiments were performed, showing just slightly different behaviour in samples with and without the peptide.

¹Kayed, R. *et al* (2009) *J Biol Chem* 284, 4230–4237.²Yanagisawa, K. *et al* (1998) *Neurobiol Aging* 19, S65–7.³Hirai, M. *et al* (2013) *Eur Phys J E Soft Matter* 36, 74.

P-490

Dynamics of amyloid proteins and their hydration water as studied by neutron scatteringK. Pounot^{1,4}, C. Marasini², Y. Fichou³, B. Vestergaard², B. Vestergaard², G. Schiro⁴, T. Seydel¹, M. Weik⁴¹Institut Laue-Langevin, France; ²University of Copenhagen, Denmark; ³University of California, Santa Barbara, USA; ⁴Institut de Biologie Structurale, France

Among the Intrinsically disordered proteins (IDP), amyloid proteins have a strong tendency to aggregate and form fibers, many of which are involved in neurodegenerative pathologies. The IDP tau and α -synuclein both form amyloid fibers consisting in cross- β structures and are implied in Alzheimer and Parkinson disease, respectively. Several studies have revealed the presence of oligomeric intermediates during the fibrillation process which could be the toxic species¹. In addition, studies on wild-type and mutant α -synuclein showed that the toxicity level is inversely correlated with the fibrillation speed, suggesting an important role of protein dynamics for the pathogenicity. Previous results from our group showed that hydration water dynamics was increased around the tau fiber core compared to the disordered monomer thereby suggesting a role of water in the fibrillation process and possibly providing a way to monitor fiber growth *in-vivo*^{2,3}. Our research aims at understanding the correlation between dynamics – of the protein itself or of hydration water – and the fibrillation process of amyloid proteins.

¹Lázaro, D. F. *et al.*, *PLoS Genet.* **10**, (2014).²Fichou, Y. *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* **112**, 6365–6370 (2015).³Schirò, G. *et al.*, *Nat. Commun.* **6**, 6490 (2015).

P-492

New insights into how calcium affects physiological/pathological function of alpha-synucleinA. D. Stephens¹, G. Fusco², N. Curry¹, A. de Simone², G. S. Kaminski Schierle¹¹University of Cambridge, UK; ²Imperial College London, UK

Alpha-Synuclein (aSyn) is a major component in familial and sporadic forms of Parkinson's disease (PD). It is a structurally disordered protein with unknown function, primarily localised in the pre-synapse. In PD the selective decline of dopaminergic neurons in the substantia nigra pars compacta may be due to their increased cytoplasmic calcium fluctuations. We aim to investigate how calcium may influence aSyn function/dysfunction.

We show that calcium binds to the C-terminus of aSyn, revealed by NMR. Biochemical assays indicate aSyn becomes more hydrophobic upon calcium binding and increases its lipid binding properties which can be reversed by the addition of EGTA. Using isolated synaptic vesicles (SV) from rat brains and NMR we reveal not only the N-terminus interacts with SV, but also the C-terminus upon calcium binding. aSyn is more prone to aggregation in the presence of SV and calcium, determined by super-resolution microscopy and ThT assays, respectively.

To conclude, calcium binding is likely important in the function of aSyn. It enhances aSyn-lipid interactions, indicating that it may have a role in endo/exocytosis of SV. However, calcium also increases the aggregation rate of aSyn, suggesting there is a fine balance between physiological and pathological function.

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– 12. Protein misfolding –

P-493

Evidence for self-replication of Alzheimer-associated A β 42 amyloid along the sides of fibrils

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The nucleation of Alzheimer-associated A β peptide monomers in a process catalysed by pre-existing A β fibrils leads to autocatalytic amplification of aggregate mass and underlies self-replication and generation of toxic oligomers associated with neurodegenerative diseases. However, the nature of the interactions between the monomeric species and the fibrils underlying this key process, and indeed the ultrastructural localisation of the interaction sites have remained elusive. Here we used NMR and optical spectroscopy to identify conditions that enable the capture of transient species during the aggregation of the A β 42 peptide. Cryo-EM images show that new aggregates protrude from the surface of the progenitor fibril. These protrusions are structurally distinct from the well-ordered fibrils observed at the end of the aggregation process. The data provide direct evidence that self-replication through secondary nucleation occurs along the sides of fibrils.

P-495

Structural comparison of peptide amyloids from human prion protein using solid-state NMR

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Proteins of diverse sequences and lengths can adopt non-native conformations and self-aggregate into amyloid fibrils. This assembling of misfolded proteins into β -rich amyloids has been linked to neurodegenerative disorders such as Alzheimer's disease and mammalian prion disease, yet amyloid-like assemblies can also serve a functional role in nature. Currently, there still lacks clear molecular details on the structural differences between pathogenic and functional amyloids to explain their divergent cytotoxicity. Here, we used solid-state NMR to examine the structure of three model amyloids formed by fragments of the mammalian prion protein (PrP) - PrP(178-183), PrP(244-249), and PrP(245-250) - previously shown to exhibit different toxicity in mammalian cell cultures. These amyloids contained similar parallel alignment of β -strands as the repeating cross- β backbone, and stacking of β -sheets to maximize burying hydrophobic side chains into a dehydrated fibril core. Residues that did not face the core were likely solvent-exposed and dictated fibril interaction with other biological entities, such as cell membrane, that lead to toxicity. This work sheds light on the effect of sequence on amyloid biology and the diverse structures of pathogenic and functional amyloids.

P-494

Size dependent structure and effects of amyloid beta42

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It has become increasingly apparent that the state of amyloidogenic aggregates is more important in neurodegenerative diseases than just their presence. The early and smaller aggregation states are thought to be most toxic to cells, but their structural properties and size are not well defined.

In this work we have used size separation via gradient centrifugation to characterise and study distinct sizes of amyloid beta42 aggregates. The use of single molecule fluorescence methods allowed us to normalize the response to relative aggregate concentration to decouple it from concentration differences. We then used various assays to study the size dependent biological effects of these aggregates; the quantitative influx of calcium into vesicles and the inflammatory response of mouse microglia cells. Interestingly we were able to detect a size dependent inflammatory response measured by TNF α ELISA.

We also characterised the aggregates using super-resolution techniques and investigated their position in the cells. This characterisation of Abeta42 aggregates of different sizes combined with the functional assays will allow us to separate the role of aggregate size and number in the response of cells to aggregates. This methodology can then be applied to other relevant protein aggregates.

Posters

– 13. Mechanosensing and mechanoregulation –

P-496 (O-81)

Binding of ZO-1 to $\alpha 5\beta 1$ regulates the mechanical properties of $\alpha 5\beta 1$ -fn linksV. González-Tarragó¹, A. Elósegui-Artola¹, E. Bazellieres², R. Oria¹, C. Pérez-González¹, P. Roca-Cusachs^{1,3}¹Institute for Bioengineering of Catalonia (IBEC), Spain;²Institut de Biologie du Développement de Marseille (IBDM), France; ³University of Barcelona (UB), Spain

Fundamental processes in cell adhesion, motility, and rigidity adaptation are regulated by integrin-mediated adhesion to the extracellular matrix (ECM). The link between the ECM component fibronectin (fn) and integrin $\alpha 5\beta 1$ forms a complex with ZO-1 in cells at the edge of migrating monolayers, regulating cell migration. However, how this complex affects the $\alpha 5\beta 1$ -fn link is unknown. Here we show that the $\alpha 5\beta 1$ /ZO-1 complex decreases the resistance to force of $\alpha 5\beta 1$ -fn adhesions located at the edge of migrating cell monolayers, while also increasing $\alpha 5\beta 1$ recruitment. Consistently with a molecular clutch model of adhesion, this effect of ZO-1 leads to a decrease in the density and intensity of adhesions in cells at the edge of migrating monolayers. Taken together, our results unveil a new mode of integrin regulation through modification of the mechanical properties of integrin-ECM links, which may be harnessed by cells to control adhesion and migration.

P-498 (O-79)

Topological defects in epithelia govern cell death and extrusionA. Doostmohammadi¹, T. B. Saw², V. Nier³, L. Kocgozlu², S. P. Thampi⁴, Y. Toyama², P. Marcq³, C. T. Lim², J. M. Yeomans¹, B. Ladoux⁵¹University of Oxford, UK; ²National University of Singapore, Singapore; ³Sorbonne Universités, France; ⁴IIT Chennai, India; ⁵Université Paris Diderot, France

Epithelia remove excess cells through extrusion, and prevent accumulation of pathological cells. Despite the important links of cell extrusion to developmental and pathological processes such as cancer metastasis, its underlying mechanism is largely unexplored. Here, we show that apoptotic cell extrusion is provoked by singularities in cell alignments in the form of comet-like topological-defects. We find a universal correlation between the extrusion sites and positions of nematic defects in the cell orientation field in different epithelium types. We model the epithelium as an active nematic liquid-crystal and compare the numerical simulations to strain-rate and stress measurements within cell monolayers. The results confirm the active nematic nature of epithelia, and demonstrate that defect-induced isotropic stresses are the primary precursor of mechanotransductive responses in cells such as YAP transcription factor activity, caspase-3 mediated cell death, and extrusions. We further demonstrate the ability to control extrusion hotspots by geometrically inducing defects through microcontact-printing of patterned monolayers. Together we propose a novel mechanism for apoptotic cell extrusion: spontaneously formed topological defects in epithelia govern cell fate.

P-497 (O-80)

Nanoscale mechanical modification in the brain tumour micro-environmentE. Minelli¹, G. Ciasca¹, G. Campi², T. E. Sassun³, M. Antonelli³, M. Papi¹, A. Santoro³, F. Giangaspero³, R. Delfini³, M. de Spirito¹¹Physics Institute, UCSC, Rome, Italy; ²IC-CNR, Monterotondo, Italy; ³Dept. of Neurology and Psychiatry and Dept. of Radiology, Oncology and Pathology, Sapienza, Rome, Italy

Glioblastoma multiforme (GBM) is an extremely aggressive brain tumour, characterized by a diffuse infiltration of neoplastic cells into the brain parenchyma. Although rarely considered, mechanical cues play a key role in the infiltration process, that is extensively mediated by the tumour microenvironment stiffness and by the occurrence of aberrant interactions between neoplastic cells and the extracellular matrix. In this work we provide the first nanomechanical characterization of the viscoelastic response of human GBM tissues by Atomic Force Microscopy. Our high resolution elasticity maps show a large difference between the biomechanics of GBM and the healthy peritumoral tissues and unveil the nanomechanical signature of necrotic regions and anomalous vasculature. Our findings open the way to the development of novel quantitative methods to assess the tumour grade, that can be used in combination with the conventional histological examinations. In order to provide a more in-depth description of the role of mechanical cues in tumour progression, we compared the nanomechanical fingerprint of GBM tissues with that of grade-I (WHO) meningiomas, a benign lesion characterized by a completely different growth pathway and a different biomechanical response.

P-499

The role of surface tension in ion channel mechanosensitivityN. Bavi^{1,2}, C. D. Cox^{1,2}, O. Bavi³, B. Martinac^{1,2}¹Victor Chang Cardiac Research Institute, Australia; ²St. Vincent's Clinical School, The University of New South Wales, Australia; ³Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Iran

Here we use the surface active agent 2,2,2-trifluoroethanol (TFE) as a pharmacological tool to study the effect of surface tension perturbations on an array of bacterial and mammalian MS channels including MscL, MscS, Piezo1 and TREK-1. We chose TFE (a general anaesthetic) because its effect on the bacterial channel MscS has already been studied. In particular TFE facilitates MscS activation from the periplasmic side, while it abolishes MscS current from the cytoplasmic side. We show that 2 % v/v TFE can also facilitate the activation of: MscL if TFE is added to either bilayer leaflet, TREK-1 only if added to the cytoplasmic side and Piezo-1 only from the extracellular side. Our molecular dynamics simulations revealed TFE increases the surface tension and the first moment of the pressure profile markedly and hence facilitates activation of MscL. Using our molecular dynamics, energetic analysis and collective experimental data, we postulate there is a relationship between MS channel shape and its activation mechanism by surface tension perturbations. Given surface active drugs are adsorbed onto cell membranes, these findings provide a mechanistic understanding of their non-specific impact on the function of various membrane proteins, particularly MS ion channels.

Posters

– 13. Mechanosensing and mechanoregulation –

P-500

Biophysical fine-tuning of immune cell behaviour by using a biomaterial-based culture platformM. H. W. Chin¹, M.-O. Coppens¹, E. Gentleman², R. M. Day¹¹University College London, UK; ²King's College London, UK

Adoptive T cell therapy is a cancer treatment that involves using in vitro-stimulated T cells to seek and destroy cancerous cells. Crucial to the efficacy of the therapy is the ability to implement in vitro activation and expansion of T cells. However, there is currently a limited repertoire of T cell-activating materials and existing ones often overlook biophysical parameters (such as substrate stiffness), which are crucial regulators of T cell activation. To address this, we propose a stiffness-tunable hydrogel platform for in vitro T cell culture. The potential impact of such a platform lies in its ability to synergistically harness biomechanical and biochemical parameters to fine-tune T cell behaviour. In this work, we have constructed T cell-activating culture substrates in a range of stiffness using polyacrylamide hydrogels coated with T cell-stimulatory antibody. Successful antibody attachment was confirmed by immunofluorescence and confocal microscopy. Also, T cells cultured on hydrogels of different stiffness exhibited a stiffness-dependent response, as indicated by their differential secretion of Interleukin-2. The preliminary findings suggest that exploiting mechanotransduction may serve as an alternative strategy to optimise T cells for immunotherapy.

P-502

Immune cell biomechanics through magnetic tweezer force studiesJ. L. Flewellen^{1,2}, P. Tolar^{1,2}¹The Francis Crick Institute, UK; ²Imperial College London, UK

Antibodies are essential to the immune response to infections. They are produced by B cells or introduced through vaccination. Pathogens with high variability, e.g. HIV and influenza, can evade the typical antibody response. However, broadly-neutralising antibodies capable of counteracting these pathogens do develop naturally, although they are usually selected against by the immune system. This selection is influenced by the physical binding between the B cell receptor and antigen proteins. A greater understanding of this binding interaction is needed to develop next generation vaccines.

We present a magnetic tweezer system and molecular biology assays to probe the mechanical interactions between HIV and B cells. We use DNA tethers and superparamagnetic microspheres along with video microscopy to facilitate high-throughput recording of many single-molecule antibody-antigen interactions simultaneously. Voice coil technology is used to move permanent magnets near the sample. The magnets can be moved over 15mm in 100ms with nanometre precision. The magnetic field introduced to the sample provides an exquisitely controllable force clamp, under which the motion of the microspheres can be captured and the biomechanics of antigen-antibody binding analysed.

P-501

HSP27 phosphorylation regulates a mechanosensitive interaction with filamin CM. P. Collier¹, T. R. Alderson¹, C. de Villiers², D. Nicholls¹, H. Gastall¹, A. J. Baldwin¹, K. Gehmlich², H. Watkins², J. L. P. Benesch¹¹Department of Physical and Theoretical Chemistry, University of Oxford, UK; ²Department of Cardiovascular Medicine, University of Oxford, UK

The small heat-shock proteins (sHSPs) are chaperones involved in stress response. The sHSP HSP27 is abundant and commonly phosphorylated in muscle tissue. HSP27 populates a large, dynamic polydisperse ensemble, making it challenging to interrogate biophysically, an experimental hurdle that is augmented when binding partners are introduced—hence, molecular details of HSP27-client interactions remain elusive. The actin-binding protein filamin C (FLNC) is a putative substrate of HSP27. Filamins play key roles in mechanotransduction and maintaining muscle integrity during mechanical stress. We show that HSP27 binds a subset of folded domains within FLNC, and localize the interaction to the region of HSP27 containing its most prevalently modified phosphosite. Using native mass spectrometry with ion mobility to probe unfolding in the gas phase, we present a possible catch-bond that is strengthened upon phosphorylation. Additional structural studies of HSP27 suggest that phosphorylation increases disorder and availability of the FLNC binding region. A protective, PTM-modulated mechanism is proposed whereby HSP27 prevents structural perturbation of FLNC from leading to full unfolding which *in vivo* could cripple its biomechanical function and lead to disease.

P-503

Dynamics of Escherichia coli's passive response to a sudden decrease in external osmolarity

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Escherichia coli has evolved ways to maintain osmotic pressure in environments that change rapidly in external osmolarity. In a situation where the external osmolarity reduces, termed hypo-osmotic shock, water rushes into the cytoplasm. To avoid bursting, cells open seven different kinds of tension-gated mechanosensitive channels (MSCs), which passively release small solutes and cytoplasmic water. Using epi-fluorescence microscopy, we monitor the volume of single *E. coli* cells when subjected to a hypo-osmotic shock of varying magnitudes. We show that the characteristic volume response consists of an expected fast expansion, on the order of few seconds, followed by a slow volume recovery, lasting up to few minutes. Using a phenomenological model we explain the observed characteristic volume response as a result of competition between water diffusing in and out of the cell, and solutes diffusing out through MSCs. However, we observed a significant cell-to-cell variability, which could be accounted for by the heterogeneity in MSC number as a result of varied pre-culturing conditions. To address this hypothesis, we investigate the volume responses of a mutant strain lacking all seven MSCs, where we control the mean number of one of the MSC by expressing them through an inducible plasmid.

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– 13. Mechanosensing and mechanoregulation –

P-504

The responses of dendritic cells to the soft fibrin hydrogel with different dimensions

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Dendritic cells (DCs) are exposed to complex biophysical force stimuli derived from different tissues and organs *in vivo* when they perform their immune functions. But the responses of DCs to the extracellular matrix with different dimension are still elusive. In present studies, DCs were seeded in the 2 and 3 dimension (2D and 3D) extracellular matrix (ECM) microenvironments (biophysical factor) made of soft fibrin gels (90Pa, without immunity). The results showed that the morphological features of imDCs and mDCs in 2D and 3D ECMs display marked differences, the F-actin cytoskeleton organizations of DCs were remodeled by 2D and 3D ECMs, the osmotic fragilities and elasticities of DCs also changed, the antigen uptaking capabilities of imDCs were decreased, moreover, some immune-associated molecules and cytokines were down- or up-regulated, indicating that the immune functions of DCs were regulated by their ECM microenvironments. These data hinted us the biophysical features (mechanical force) of ECM microenvironments should be considered when investigating microenvironment biophysical factors-sensitive immune cells in order to exactly understand their immunological functions. Also, our data supported the viewpoint of immuno-mechanobiology or mechano-immunology.

P-506

Biophysics of force sensing kinasesC. Kluger^{1,2}, S. M. Sedlak^{1,2}, M. S. Bauer^{1,2}, F. Baumann^{1,2}, L. C. Schendel^{1,2}, D. A. Pippig^{1,2}, H. E. Gaub^{1,2}¹Chair of Applied Physics LMU München, Germany; ²Center for NanoScience (CeNS) LMU München, Germany

Proteins inside living organisms are not only involved in biochemical signaling cascades but also subjected to mechanical forces. Mechanically induced conformational changes in protein structures can reveal new binding sites for interaction partners and expose surfaces needed for enzymatic reactions. This mechanoregulation is especially important for cellular adhesion and in muscle structures like the sarcomere.

One example for mechanoenzymatic behavior in muscle tissue is the giant elastic protein titin that contains a kinase domain that has been shown to be activated as response to strain. Here, we show a similar mechanism of force activation for myosin light chain kinase (MLCK) and discuss first results on the mechanical unfolding of focal adhesion kinase (FAK).

By combining AFM-based force spectroscopy with single molecule readout of protein phosphorylation, new insights into the spatial and temporal regulation of kinase activity are gained. The use of in-vitro expression technologies and site-specific covalent surface attachment further enables us to generate higher throughput to screen for the influence of disease related mutations or isoform specific mechanical properties.

P-505

Tension generation in epithelial cells - the impact of a single cell defect

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For epithelial cells which are lining organs or cavities, the mechanical integrity of a whole cell cluster is indispensable. Therefore, rapid wound closure after injury is a very crucial process for maintaining the barrier function of these cells.

In this study, single cell wounds are induced in an intact layer of MDCK II cells.

The closure process is monitored by microscopy to characterize the exact closing mechanism. It turned out that the induced defect is compensated quite rapidly by transporting the defect cell out of the layer with a combination of acto-myosin contractility and migration. Furthermore, cells neighboring the wound were mechanically characterized. Changes in the pretension of the apical cell cortex and in membrane tension became visible. This specific mechanical response was detected not only in direct neighbors of the defect but also in cells further away. This implies that the layer shows a collective mechanical response to a comparable small disturbance.

P-507

Oxygen gulp in microwounded cells of *Chara corallina* detected by novel O₂ nanosensorsA. Komarova¹, P. Gorelkin², A. Erofeev¹, T. Bibikova¹, Y. Korchev³, A. Bulychev¹¹Lomonosov Moscow State University, Russian Federation; ²OOO "MNT", Russian Federation; ³Imperial College London, UK

Molecular oxygen plays a crucial role in plant metabolism. O₂ is a source of reactive oxygen species (ROS) and oxidative stress. The excessively generated ROS play both signaling and protective function in mechanically stressed plants. Generation of ROS in microwounded cells is presumably mediated by the plasma membrane NADPH-oxidase that transfers electrons from cytoplasmic NADPH to extracellular oxygen with a concomitant production of ROS, H₂O₂ in particular. Microscopic injuries associated with ROS generation might be accompanied by oxygen concentration changes in the apoplast. Recent invention and elaboration of nanoscale electrochemical sensors provide the opportunity to test this hypothesis. Our data obtained by applying carbon-filled quartz micropipettes with platinum-coated tips (oxygen nanosensors) showed a considerable drop in oxygen concentration at *Chara corallina* cell surface in response to microperforation of the cell wall (CW). We tested possible involvement of the suppression of photosynthesis, the enhancement of respiration, and the activation of the plasma-membrane NADPH oxidase as an origin of oxygen decline upon CW microwounding. The results provide evidence for major role of plasmalemmal NADPH-oxidase in the discovered local drop of O₂ content.

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– 13. Mechanosensing and mechanoregulation –

P-508

Mechanosensing and dynamics of cell filopodiaO. Mikhajlov¹, G. Tran van Nhieu², P. Bassereau¹¹Laboratoire Physico Chimie Curie, Institut Curie, CNRS UMR168, 75248 Cedex 05 Paris, France, France; ²Equipe Communication Intercellulaire et Infections Microbiennes, Centre de Recherche Interdisciplinaire en Biologie, Collège de France, Paris, France, France

Filopodia are very thin dynamic tubular protrusions at cell edge that allow cells to sense their surrounding and exert traction forces. They are involved in the migration of healthy cells as well as of metastatic cancer cells. Filopodia are also hijacked by pathogens during cell invasion. How filopodia sense the mechanical and biochemical properties of their environment is still unclear. Among many proteins involved in this process integrins, transmembrane receptors, play an important role. Being a bridge between an extracellular matrix and cytoskeleton they can be activated by various signals (mechanical or chemical) and transduce them from the outside to the inside of the cell. Activated integrins can cluster on the cell membrane, which leads to an amplification of a downstream signal.

The general objective of this work is to induce integrin clustering and study the cell's mechano-response in a controlled manner using advanced fluorescence microscopy coupled with optical tweezers. Integrin clustering can be achieved by contact with integrin ligands either immobilized on a bead or freely diffusing on a giant vesicle. In this case, the vesicle itself is used as a force transducer with stiffness adjustable through membrane tension. Clustering of integrin and of its downstream partners is optically detected.

P-510

Mechanosensitivity of polydiacetylene with a phosphocholine headgroup

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Membrane forces play pivotal roles in cell response. Current characterization methods allow for controlled force application but not for detection of forces applied to the bilayers. Conversion into local forces is difficult. We are developing a mechanosensitive membrane probe that allows direct measurement of forces within lipid bilayers. We employ a mechanochromic polydiacetylene polymer (PDA) that changes color and fluorescence intensity upon application of forces. We demonstrated that a PDA vesicle assay detects melittin, a transmembrane peptide. First we fabricated PDA vesicles and crosslinked the monomers by UV. PDA vesicle suspension absorption spectra was found to shift over the next 4 hours following melittin addition. The spectra changed completely as the color change was also clearly visible by eyes. The developed assay proved that DiynePC PDA is sensitive to mechanical stimuli with the advantage of its phosphocholine head group that mimics cell membranes. The combination of sensitivity and biocompatibility will allow to incorporate this probe in live cells, providing further possibility to investigate force distribution during the cell life cycle and its interactions with medical implants.

Ortuso, R. D.; Cataldi, U.; Sugihara, K. *Soft Matter* **2017**, 13, (8), 1728–1736.

P-509

Utilizing the osmoregulatory network of E.coli to control mechanically induced gene expression

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Bacteria regulate the expression of their genome in response to a wide range of environmental conditions, including temperature, external osmolarity, presence or absence of certain chemical species and density of neighbouring cells. However, and in contrast to mammalian cells, there remain relatively few examples of changes in bacterial gene expression directly as a result of applied external mechanical forces. To achieve genetic expression of a fluorescent reporter protein, in response to controlled mechanical compression, we use a part of *E. coli*'s osmoregulatory network which is up-regulated at higher osmolarities to produce trehalose, which helps to maintain *E. coli*'s volume and osmotic pressure. Here we present a custom microscopy and microfluidic platform we developed that allows us to control the application of mechanical force to single *E. coli*'s cells simultaneously with imaging. Using the platform and the reporter fluorescent protein, expressed both constitutively on the chromosome and on a plasmid, we investigate the link between mechanical forces and gene expression, with a view to developing a whole cell mechanical force biosensor.

P-511

The influence of neighboring cells on elasticity of single cells measured by atomic force microscopy

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The environment surrounding cells influence cellular response to mechanical stimuli, which is manifested as changes in their mechanical properties. Various studies have reported that the elasticity of single cells can be used as a marker of distinct disease progression, including cancer. The main objective of our studies was to quantify elasticity changes induced by the presence of neighboring cells of the same and different type. In a system, being a co-culture of human skin fibroblasts (FBs) and keratinocytes (HaCaT), FBs appeared to be more sensitive to the presence of neighboring cells as compared to HaCaT cells. These results were driving force to estimate cellular deformability in a system composed of HaCaT and melanoma cells (WM35).

The JPK AFM purchase has been realized under the project co-funded by the Malopolska Regional Operational Program, Measure 5.1 – “Krakow Metropolitan Area as an important hub of the European Research Area” for 2007-2013.

Posters**– 13. Mechanosensing and mechanoregulation –****P-512****Towards simultaneous force and fluorescence spectroscopy**L. C. Schendel^{1,2}, M. S. Bauer^{1,2}, S. M. Sedlak^{1,2}, F. Baumann^{1,2}, C. Kluger^{1,2}, D. A. Pippig^{1,2}, H. E. Gaub^{1,2}¹Chair of Applied Physics LMU München, Germany; ²Center for NanoScience LMU München, Germany

In the past years, Zero-Mode Waveguides (ZMWs) have emerged to a powerful application in modern life science. They provide subdiffraction detection volumes in single-molecule fluorescence measurements and hence experimental performance at physiological concentrations of fluorescently labeled biomolecules.

Here, Atomic Force Microscopy and ZMWs are combined in order to investigate force-activation pathways of enzymes. For this purpose, high concentrations are dictated by experimental conditions and requirements, this being high Michaelis-Menten constants of enzymes and the limited time span of keeping the protein's binding pocket open/accessible. Although force-triggered ATP binding events were already investigated for Titin Kinase with simultaneous force fluorescence spectroscopy, the experimental yields of these events remained low. However, they are expected to increase by implementing a covalent site-specific chemistry together with an optical non-invasive cantilever localization routine. In the future, this improved methodology will be applied to investigate also further enzymes upon possible force-activation mechanisms.

Along with ongoing development in protein Single Molecule Cut and Paste, this leads the way for future investigations on enzymatic networks inside ZMWs.

P-514**Modeling fiber interface with stochastic cross-bridges**F. Wijanto^{1,3}, M. Caruel², J.-M. Allain^{1,3}¹Ecole Polytechnique, France; ²Université Paris-Est, France; ³Inria, France

Biological organisms make extensive use of fiber networks for structural integrity across multiple length scales. In this study, we develop a stochastic fiber network model, where the mechanical response of interacting fibers is given by stochastic attachment and detachment of springlike cross-bridges.

Rigid filaments are mechanically coupled via parallel springs possessing on one hand a stochastic attachment rate, dependent on the distance to binding sites on the filaments and on the other hand a stochastic detachment rate, dependent on local mechanical load.

The response exhibits two features separated in time scales. For long time scales, a stable steady-state force appears, akin to viscous drag, but which depends non-monotonously on the sliding speed. This shows that an optimal sliding speed exists with respect to maximal adhesion between fibers.

At short time scales, avalanche-like fluctuation patterns due to cascading detachment events are observed, which persist throughout the steady state.

Our model thus shows that applying stochastic attachment/detachment events can cause the emergence of viscous non-linear behavior from purely linear mechanical elements.

P-513**Carbon nanotubes scaffold influences the onset of calcific aortic valve disease**L. Ulloa Severino^{1,2}, I. Rago^{1,2}, F. Perissinotto^{1,2}, R. Santoro³, M. Pesce³, L. Casalis², D. Scaini^{1,2}¹University of Trieste, Doctoral School in Nanotechnology, Trieste, Italy; ²Elettra-Sincrotrone S.C.a.p., Trieste, Italy; ³Centro Cardiologico Monzino, IRCCS, Milano, Italy

Calcific Aortic Valve Disease is the most common form of valve disease in the Western world and represents a major healthcare burden. The primary driver for valvular calcification is the differentiation of valvular interstitial cells (VICs) into a diseased phenotype, the osteoblastic-like cells. Another feature of this disease is the significant change in the organization, composition and mechanical properties of the extracellular matrix that it seems to contribute to the progression of the pathology altering cellular molecular signaling. In recent years, the quest for biocompatible materials capable of mimicking the natural ECM for tissue regeneration has increased. Carbon nanotubes (CNTs) are optimal candidate in this context, showing dimensions comparable to fibrillar ECM constituents, in-vitro and in-vivo cellular biocompatibility. In the present study, we studied the mechanical, morphological and molecular properties of VIC grown on randomly oriented CNTs. Using the Atomic Force Microscopy and immunofluorescence microscopy we assessed the biomechanics, morphology and the pathway involved in the mechanical stress and in onset of pathology. Our results identified CNTs as good materials to use for engineering aortic valve artificial scaffolds.

P-515**Orchestration of Mechanotransduction Machinery in Cochlear Hair Cells**

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Physical and chemical signaling pathways are the two major type of sensations from cell-cell communication to organism survival all over the world. While mechanosensation and mechanotransduction are least understood among those physical sensations, including mechanical signal transduction and transducer channel mechanisms. Here we use cochlear hair cells to study mechanotransduction that rapidly converts sound induced mechanical vibrations into electrical signals. With limited annotation from genetic study, we have known half dozen of genes that encode components critical for mechanotransduction. We further investigated the hair bundle anatomy, transducer current kinetics, channel-gating properties in normal hair cells and mutant hair cells. More interestingly, the transducer complex also possess altering properties in topographic fashion along the cochlear coil with currently unknown mechanisms. In summary, we are trying to understand transducer machinery from molecular mechanism to hair cell function and to cochlear physiology.

Posters**– 13. Mechanosensing and mechanoregulation –****P-516****Biophysical Studies on Dendritic Cells**

Z. Zeng

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DCs-based immunotherapy against cancer is considered one of the most promising therapies to overcome cancers, but there are still many challenges need to be overcome. This study focuses on the investigations of DCs at different differentiation stages and under various tumor microenvironments as well as extracellular matrix from the interdisciplinary viewpoints. The results showed that the DCs at different differentiation stages appear various biophysical characteristics. The tumor microenvironment-derived cytokines impair the biophysical properties of DCs, moreover, these changes are closely correlated with the expression levels of some cytoskeleton-binding proteins. The immune functions of DCs could be regulated by physiological fluid flow and extracellular matrix-derived mechanical forces, supporting the viewpoints of mechanoimmunology. It is significant for further understanding of the biological behaviors of DCs and improving the clinical therapy efficiency of DCs-based immunotherapy against cancers.

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Posters

– 14. Correlative, multiscale and functional imaging –

P-517 (O-85)

Using STORMForce for understanding how bacteria grow and die

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AFM is able to achieve nanometre resolution, and to sensitively measure forces to study molecular and cellular processes. However AFM is only able to do this on the surface of a sample, where STORM uses fluorescent labels to carry out sub-optical diffraction limit imaging. This enables the user to label specific proteins within the membrane whilst force mapping or imaging the environment that it is in. Here we studied the cell wall of *E. coli* bacteria. The details of the architecture and the way in which that architecture is formed are still poorly understood.

Firstly a STORMForce image was obtained through imaging on separate equipment and overlaying the images. A protocol has been developed that is suitable for both AFM and STORM.

For an integrated STORMForce image an AFM image was taken 1st and then a STORM image was obtained from the same area. Progress towards molecular resolution AFM using “Hyperdrive” with molecular localisation using STORM on the combined STORMForce instrument will be presented.

P-519 (O-87)

Cell-temperature mapping by Eu-doped TiO₂ nanothermometers

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Temperature regulates many biomolecular reactions within cells. Measuring of temperature on a nanometer scale has therefore presented a desirable challenge for the last two decades. Several biocompatible nanothermometers have been proposed as potential sensors, including lanthanide nanoparticles, quantum dots, gold nanoparticles, fluorescent polymers, and polymers with encapsulated fluorophores. To overcome the problem related to possible temperature gradients in a cell, precise nano-scale temperature measurements has been done also in this work using Eu³⁺-doped TiO₂ nanospheres. This material is known to adhere to membranes and can even be internalized. Our experiments clearly show that a temperature increase of few K can be easily detected. Temperature measurement with the Eu³⁺-doped TiO₂ nanospheres should be artifact-free, since Eu³⁺ atom is incorporated in a TiO₂ lattice, and thus isolated from intracellular environment. On the other hand, the sensor is still very small, with a size of few nanometers only enabling to detect local temperature gradients if they exists.

P-518 (O-86)

Study on acoustic signal features influenced by thermoacoustic effects in magnetoacoustic tomography

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Magnetoacoustic tomography (MAT) is an emerging non-invasive electrical conductivity imaging method that combines the high dielectric contrast of tissues and excellent resolutions of ultrasonography, which is a promising modality for tumor early diagnose. In our study, we have found that the thermoacoustic (TA) effect occurred in coincidence with the measurement of acoustic signal in MAT. In order to investigate the influence of the TA effect on the acoustic signal, experiments on several materials with different conductivities were conducted by a MAT system both with and without a static magnetic field. The acoustic signals were analyzed then compared in the time domain and the frequency domain, respectively. The TA effect is found to be related to material characteristics. For tissue-like materials with low conductivities, the TA signals caused by the TA effect are observable and cannot be ignored in both domains. This demonstrates that the TA effect occurred in MAT would influence the acoustic signal features. More rigorous algorithms should be developed for the MAT system in the future, to obtain better imaging quality.

P-520

Correlative nanoscopy on the study of supramolecular assemblies

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A wide range of human diseases (e.g. Alzheimer's and Parkinson's diseases) arises from uncorrected protein folding and subsequent aggregation, giving rise to the formation of large fibrillary aggregate, called amyloid fibrils. A key requirement to understand the molecular mechanisms of these diseases is the capability to characterize the aggregation processes in its different steps, also observing the corresponding structures formed *in vitro*. A new and original approach in the study of the fibrillation processes is to apply advanced integrated systems obtained by coupling a standard atomic force microscope (AFM) and super-resolution optical microscopes.

We studied the simultaneous aggregation of the two model proteins, insulin and lysozyme, in the same environmental conditions. Proteins monomers were labelled in order to track fibrillar growth by using AFM together with stimulated emission depletion (STED) microscopy. The coupled system allows to obtain super-resolution fluorescence images, perfectly overlapped with AFM topography and with a highly localized chemical recognition capability. This new kind of analysis suggested some important cues on the mechanisms followed by proteins in their aggregation pathway.

Posters

– 14. Correlative, multiscale and functional imaging –

P-521

3D tomographic imaging of biological objects using hard X-ray Bragg magnifier microscopeS. Hrivnak¹, J. Ulicny¹, L. Mikes¹, P. Vagovic^{2,3}¹Center for Multimodal Imaging (CMI), Department of Biophysics, Faculty of Science, P. J. Safarik University, Jesenna 5, 04154 Kosice, Slovakia; ²European XFEL, Albert Einstein Ring 19, 22761 Hamburg, Germany; ³Center for Free Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany

We present an alternative method for 3D tomographic imaging of microscopic biological objects in hard X-ray regime, based on Bragg Magnifier (BM) principle. BM microscope uses asymmetrically cut Germanium crystals to magnify X-ray beam with advantages such as shorter propagation distances and increased dose efficiency, while achieving decent spatial resolution. This work focuses on the 3D reconstruction method to interpret the X-ray tomographic holograms using single-distance phase retrieval algorithm developed specifically for Bragg Magnifier, which is followed by filtered back-projection. We use a modification of contrast transfer function approaches developed for propagation based phase-contrast imaging and in combination with iterative constraint-based phase retrieval algorithm we obtained faster and more robust reconstruction method. Our algorithm was successfully applied to both synthetic and real-world experimentally measured holograms as demonstrated on 3D electron density reconstruction of model organism *Tardigrade*. We reached isotropic spatial resolution 300 nm approaching theoretical resolution limit for the given experimental setup.

P-523

Pulse-shaped multiphoton excitation of single molecules

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Multiphoton excitation could have advantages over resonant excitation for single molecule spectroscopy. For example: deeper penetration of biological tissue due to reduction of out of focus excitation, access to different excited states, large spectral separation of excitation and emission and broad excitation spectra [1]. We seek to use the next generation of ultrafast lasers for single-molecule detection. However, dispersion from optical components can limit their use.

In this work we will describe a new home-built multiphoton fluorescence setup and present preliminary measurements. It deploys an ultra-broadband Ti:Sapphire laser (bandwidth 135 nm FWHM) and a pulse shaper for pre-compensation of broadening using the MIIPS-method [2, 3]. First experiments show that it is possible with this setup to achieve pulses of 9fs length in the focal plane of the microscope.

[1] He, G. S. *et al.*, Chem. Rev., **108**, 1245–1330 (2008)[2] Coello, Y. *et al.*, JOSA B, **25** A140–A150 (2008)[3] Lane, R. S. K. *et al.*, Opt. Express, **20** 25948–25959 (2012)

P-522

Correlated cryo-fluorescence and cryo-electron microscopy can identify sites of membrane fusionL.-A. Metskas^{1,2}, J. A. G. Briggs^{1,2}¹MRC Laboratory of Molecular Biology, Cambridge, UK;²European Molecular Biology Laboratory, Heidelberg, Germany

Advances in cryo-electron microscopy have recently been coupled with correlated light and electron microscopy (CLEM), where fluorescence is used to locate regions of interest prior to data collection. While the goal of cryo-CLEM is typically localisation, the fluorescence can also probe function or dynamics if conditions are optimised for cryo-fluorescence microscopy. In this way, cryo-CLEM has the ability to assist not only in efficient high-resolution structural study, but also a description of the functional state of the molecule(s).

During membrane fusion, two bilayers enter into apposition, then progress to hemifusion and finally full fusion; this can be monitored by tracking dequenching of a concentrated lipid dye upon hemifusion with an unlabelled vesicle. We have identified conditions under which this assay can be performed on a cryo-CLEM grid, allowing localization based on lipid mixing, or unambiguous determination of lipid mixing in micrographs where this is not clear based on visualization alone. We have applied this method to a study of influenza virus-like particles fusing with synthetic lipid vesicles. We are adapting our cryo-CLEM system [1] to improve the signal-to-noise ratio and facilitate automation.

[1] Schorb, M. *et al.* (2016) *J. Struct. Biol.* 197(2): 83–93.

P-524

Image Mean Square Displacement analysis: a new method to study protein diffusion in cell membranesE. Rao¹, M. A. Digman^{2,3}, V. Vetri^{1,4}, E. Gratton²¹Dept. of Physics and Chemistry, University of Palermo, Italy; ²Laboratory for Fluorescence Dynamics, Dept. of Biomedical Engineering, UC Irvine, USA; ³Dept. of Chemical Engineering and Material Sciences, UC Irvine, USA;⁴Aten Center, University of Palermo, Italy

The Image Mean Square Displacement (iMSD) analysis is one of the newest techniques developed to investigate the diffusion of proteins within live cells. Based on the concepts of Fluorescence Correlation Spectroscopy and in particular on Spatiotemporal Image Correlation Spectroscopy method, iMSD analysis allows monitoring molecular dynamics obtaining protein diffusion laws. Starting from a stack of fast-acquired images, the new analysis technique gives the opportunity to map the diffusion of proteins in the entire cell, identifying the regions in which the protein is undergoing pure isotropic, confined, transiently confined or directed motion. As a result, maps of diffusion modes, of diffusion coefficients and of the size of fluorescent proteins diffusing inside the region are obtained, as well as local values for all the parameters characterizing the motion. The great potential of iMSD method is that diffusion laws can be obtained directly from imaging, in the form of an iMSD vs time-delay plot. Total Internal Reflection Fluorescence (TIRF) microscopy images have been analyzed to investigate the diffusion of Epidermal Growth Factor Receptor in live cell membranes, before and after stimulation with Epidermal Growth Factor.

Posters**– 14. Correlative, multiscale and functional imaging –****P-525****Dynamics of peroxisomes - from protein to organelle level**K. Reglinski¹, S. Galiani¹, D. Waithe², J. Klümper³, W. Schliebs³, R. Erdmann³, C. Eggeling¹¹MRC Human Immunology Unit, WIMM, University of Oxford, UK; ²Wolfson Imaging Centre, WIMM, University of Oxford, UK; ³Institute of Physiological Chemistry, Systemic Biochemistry, Ruhr-University Bochum, Germany

Peroxisomes are cell organelles with a size of 0.3–0.6 µm in mammalian cells, fulfilling many different functions like the oxidation of fatty acids, detoxification of reactive oxygen species as well as the biosynthesis of different lipids. The import of proteins into peroxisomes differs from import processes of other organelles, since peroxisomes import already folded and even oligomerized proteins from the cytosol. Proteins destined for the peroxisomal matrix are recognized by the peroxisomal import receptor PEX5, which binds them in the cytosol and directs them to the peroxisomal membrane. Here PEX5 binds to peroxisomal membrane proteins and integrates into the membrane, forming a transient translocation pore through which the cargo proteins are imported. Here we present our advanced microscopy experiments to characterize the dynamics of this import process: (1) Super-resolution STED microscopy to highlight compartmentalized protein distributions at the peroxisomal membrane; (2) Fluorescence Correlation Spectroscopy (FCS) to analyse the interaction dynamics between PEX5 and its cargo proteins; and (3) tracking the movement of peroxisomes to understand the molecular mechanisms that trigger their mobility.

P-527**Chromatin dynamics and viscoelasticity are determined by lamin A interconnections**F. Taheri¹, L. Lau¹, G. Marcarini², B. Isbilir¹, G. Chirico², J. Langowski¹¹DKFZ, Biophysics of Macromolecules, Heidelberg, Germany; ²University of Milano-Bicocca, Physics Dept., Italy

Using fluorescence correlation spectroscopy in single plane illumination (SPIM-FCS), we investigated the dynamics of chromatin in interphase mouse adult fibroblast (MAF) cell nuclei under the influence of the intermediate filament protein lamin A. We find that a) lamin A-mRFP and histone H2A-eGFP show significant co-mobility, indicating strong interaction inside the nucleus, and b) that the random motion of the chromatin network is subdiffusive, i.e., the effective diffusion coefficient decreases for slow time scales. Knocking out lamin A changes the diffusion back to normal. Thus, lamin A influences the dynamics of the entire chromatin network. Our conclusion is that lamin A plays a central role for determining the elasticity of the chromatin network and to help maintaining local ordering of interphase chromosomes.

P-526**Ratiometric imaging with super-resolution STED microscopy to reveal nanoscale membrane heterogeneity**

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The lateral organization of molecules in the cellular plasma membrane plays an important role in cellular signaling. A critical parameter for membrane molecular organization is how the membrane lipids are packed. Polarity sensitive dyes are powerful tools to characterize such lipid membrane order. These dyes change their emission spectrum depending on the polarity of the environment which can be used to quantify the molecular ordering and to visualize lateral heterogeneity in membrane order of cellular membranes. These probes have been used in combination with confocal or multi-photon microscopy; however, the diffraction-limited spatial resolution of these techniques does not allow observation and full characterization of nanodomains/clusters in the plasma membrane. The investigation of potential lipid nanodomains, however, requires the use of super resolution microscopy. Here, we apply the polarity sensitive membrane dyes in super-resolution STED microscopy. Measurements on cell-derived membrane vesicles, in the plasma membrane of live cells, and on single virus particles show the high potential of these dyes for probing nanoscale membrane heterogeneity.

P-528**Developing probes for cryo-superresolution light and electron microscopy**M. W. Tuijtel¹, M. Bárcena¹, A. J. Koster^{1,2}, F. G. A. Faas¹, T. H. Sharp¹¹Leiden University Medical Center, Netherlands; ²NeCEN, Netherlands

Correlative light electron microscopy (CLEM) combines the ability of fluorescence light microscopy (FLM) to locate rare or transient cellular events within a large field of view with the high spatial resolution of electron microscopy (EM). CLEM is therefore a powerful technique to study cellular processes. The development of probes that are both fluorescent and electron dense increases the applicability and usability of CLEM by improving correlation accuracy and simplifying sample preparation. High accuracy correlations between FLM and EM images are critical to maximize the use of super-resolution light microscopy as a tool for CLEM. We present our work developing a dual-purpose stain for cryo-FLM and EM of high-pressure frozen and resin-embedded cellular sections that simplifies correlation and increases alignment accuracy, as well as developing the use of fluorescent probes for super-resolution cryo-CLEM.

Posters

– 15. Evolution, ecology, collective and emergent behaviour –

P-529 (O-91)

Dynamics of bacterial community architecture governs viral protection and dispersal mechanisms

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In nature, bacteria primarily live in communities, termed biofilms that have many emergent properties. In medical settings, biofilms cause devastating damage during infections; indeed, bacteria are often viewed as agents of human disease. However, bacteria themselves suffer from diseases, mostly in the form of viral pathogens termed bacteriophages, which are the most abundant replicating entities on Earth. Phage-biofilm encounters are extremely common in the environment, but the mechanisms that govern these interactions are unknown. Using *E. coli* biofilms and the lytic phage T7 as models, we discovered that an amyloid fiber network protects biofilms against phage attack *via* two separate, novel mechanisms. First, collective cell protection results from inhibition of phage transport into the biofilm. Second, amyloid fibers protect cells individually by coating their surface and binding phage particles, thereby preventing their attachment to the cell exterior. We also discovered that the matrix dynamics governs the dispersal process of cells from biofilms. These insights into biofilm-phage interactions and matrix architecture have broad-ranging implications for phage applications in biotechnology, phage therapy, and the evolutionary dynamics of phages with their bacterial hosts.

P-531 (O-93)

Collective feeding in *C. elegans*

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Collective behaviour is frequently studied in groups of large animals but is less well understood in small organisms and at mesoscopic scales. Here we investigate the collective feeding behaviour of the nematode worm *C. elegans*, well known for its easy genetic manipulability and stereotypic, yet complex behaviour.

By tracking many worms and probing the dynamics inside aggregates with fluorescent imaging we quantify behavioural differences between the “asocial” lab strain, “social” wild isolates, and an aggregating mutant strain.

Drawing on concepts from motility-induced phase transitions in active matter and bacterial systems, we interrogate the mechanism of aggregation by quantitative analysis of tracking data and computational modelling of worm movement. Our study elucidates how small genetic differences affect the emergent phenotypes of group behaviour, and how social information flows from individuals to the population.

P-530 (O-92)

Stochasticity and division of labour in toxin production in two-strain bacterial competition in *E. coli*

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Interactions within bacterial communities play a crucial role in community composition dynamics and maintenance of biodiversity. Here we show that competition of an *E. coli* strain C, which performs division of labor between reproducers and self-sacrificing toxin producers, with a toxin sensitive strain S, can lead to four different outcomes: dominance of S or C, coexistence, and extinction of both strains. To disentangle the impact of division of labor and stochasticity in toxin production on competition and C strain success, we employed an experimental approach that permits observations of the competition at multiple length-scales (near single cell to macroscopic levels). We found that the stochastic toxin production dynamics affect the competition twofold. First, in the initial phase ($t < 12$ h), it influences the formation of viable C clusters at the colony edge. Second, it determines the toxin producer fraction within the C population, which dictates the deterministic competition dynamics in the second phase. In addition, we developed a stochastic and spatially extended computational model of the competition to complement the experiments. Our findings enhance the understanding of division of labor and the importance of stochasticity in toxin production for bacterial competition.

P-532

Trapping a single bacterial cell and its progeny to study the emergence of phenotypic heterogeneity

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Despite their genetic identity cells in a clonal population exhibit a variation in their phenotypic characteristics. This heterogeneity contributes to the fitness of the population especially in a changing environment. Such variability have been observed for various different cell types, from bacteria to mammalian cells. However, the origin and the processes behind the emergence of phenotypic heterogeneity remain largely unknown.

We have developed a microfluidic device consisting of an array of single cell traps to study the emergence of phenotypic heterogeneity in bacteria. Following division of a trapped cell one daughter cell remains in the trap while the other drops out and falls into the next empty trap. After several divisions all the traps are filled with the progeny of a single cell. All trapped cells may be imaged and characterized using high resolution microscopy. Phenotypic characteristics such as cell size, cell shape, division rate or gene expression levels may be precisely measured in an experimental timeframe reaching 100 generations. By analyzing this data along with the information on the relatedness of each cell we are exploring the basic principles and processes behind the emergence of phenotypic heterogeneity in a population originating from a single cell.

Posters

– 15. Evolution, ecology, collective and emergent behaviour –

P-533

Antibodies adhesion and mechanical forces select for moderate bacterial growth rate in the gut

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Immunoglobulin A are antibodies produced by the adaptive immune system and secreted in the gut lumen to fight pathogenic bacteria. It has been recently shown that a main physical effect of these antibodies is to enchain daughter bacteria, i.e. to make that dividing bacteria are likely to remain agglutinated. When in clusters, bacteria are less motile and cannot interact with the epithelial cells. We model the dynamics of these clusters. Adhesion between bacteria will break at a certain rate. Using analytical models and simulations, we show that for a range of parameters, the rate of increase in the number of free bacteria (which can interact with the epithelium) has a maximum as a function of the replication rate of bacteria. At low replication rate, the faster the replication, the more the increase of free bacteria. But at higher replication rate, the bacteria replicate before the adhesion between daughter bacteria breaks, leading to growing cluster size and preventing bacteria from escaping the clusters. This enable the gut to select against fast replicating bacteria, which could destabilize the microbiota, and which are more likely to be pathogenic. We also study the evolutionary consequences of the clonality of these clusters.

P-535

Microscale bacteria-oil interactions in bioremediationV. I. Fernandez¹, G. Juarez², R. Stocker¹¹ETH Zurich, Switzerland; ²University of Illinois at Urbana-Champaign, USA

Dispersants are used to break up marine oil slicks and increase the available surface area for bacteria to degrade oil hydrocarbons. However, this argument neglects key elements of the microscale interaction between bacteria and oil droplets, namely encounters, surface attachment, and growth. Utilizing experimental observations of bacteria colonizing oil droplets, we model the interactions affecting hydrocarbon consumption between a collection of oil droplets and a single bacterial pool. The results indicate that degradation time is minimized for intermediate droplet sizes, as maximizing the surface area to volume ratio is countered by increased encounter times under most conditions. Oil degradation times and the optimal droplet size are also strongly dependent on oil concentration, but comparatively insensitive to starting bacterial concentrations. In poly-dispersed droplet distributions, larger oil droplets can significantly increase the overall degradation rate by driving temporary reductions in bacterial encounter rates. This mechanical model provides a baseline for understanding oil biodegradation and mitigation strategies in open marine systems, and highlights pitfalls in laboratory oil biodegradation experiments.

P-534

Studies on the bioremediation properties of amino acid-based surfactants and rhamnolipidsB. Come¹, D. R. Perinelli², G. F. Palmieri², R. Itri³, M. da Silva Baptista⁴, L. Santos⁴, P. Mariani¹, F. Spinozzi¹¹Department of Life and Environmental Sciences, Marche Polytechnic University, Italy; ²School of Pharmacy, University of Camerino, Italy; ³Institute of Physics, University of Sao Paulo, Brazil; ⁴Institute of Chemistry, University of Sao Paulo, Brazil

We present a structural and toxicity study of two classes of surfactants for bioremediation processes: new synthesized amino acid-based surfactants [1] and rhamnolipids [2].

Both classes of surfactants were investigated by the X-ray diffraction technique. Interaction with membranes were studied using Giant Unilamellar Vesicles (GUVs) as model membranes and performing viability assays in HaCAT cell cultures. GUVs were prepared by electroformation method. HaCaT is a spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin. The obtained results showed that N-decanoyl amino acids (1mM) and Rhamnolipids (1mM) cause damage to the membranes of the GUVs. The lipid bilayer initially presents an excess of area resulting in the formation of tubes and/or buds that remain linked to the original membrane. Cell viability is around 20% at concentrations of rhamnolipid around 400µM.

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[2] J.D. Desai, I.M. Banat, Microbiol. Mol. Biol. Rev. 61 (1997) 47

P-536

Origin of multicellularity: evolution of increased size via improved cellular packing efficiencyS. J. Jacobeen¹, J. T. Pentz², E. Graba¹, C. Brandys¹, W. C. Ratcliff², P. J. Yunker¹¹School of Physics, Georgia Institute of Technology, USA;²School of Biological Sciences, Georgia Institute of Technology, USA

The evolution of multicellularity transformed life on earth—both by providing its bearers with immediate fitness advantages, and by setting the stage for profound increases in organismal complexity. However, these advances came at a price: even before the development of regulatory networks, bodies composed of multiple cells must contend with previously irrelevant forces capable of breaking intercellular bonds. Using the snowflake yeast model system, I examine the physical underpinnings of early evolution in nascent multicellular clusters. Snowflake yeast reproduce when internal stress from growth causes the cluster to fracture into independently viable propagules. Under daily selection for large size, snowflake yeast evolve improved fitness by increasing their mean size at fracture. This is achieved by increasing cellular aspect ratio, which improves packing efficiency within the cluster and thus delays fracture by decreasing the rate of internal stress accumulation. Geometric simulations confirm that the observed increase in cellular aspect ratio substantially increases the number of growth configurations – cell placements that won't cause fracture – accessible to a cluster.

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Liquid ordered phase formation by membrane sterols was required for eukaryotic membrane development

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The major sterols in the plasma membrane of fungi and mammalian cells – ergosterol (Erg) and cholesterol (Chol), respectively – share zymosterol (Zym) as a common metabolic precursor. Here, a transversal approach from *in vitro* model systems to living yeast and mammalian cells was used to assess the ability of these metabolically related sterols to form liquid ordered (l_o) lipid domains and modulate several biophysical properties of lipid bilayers. We unequivocally show that one major property is shared by Erg and Chol and can be directly correlated to their ability to form l_o phase: these sterols preferentially interact with saturated vs. unsaturated phospholipids. In contrast, Zym is unable to form l_o phase with either type of phospholipid, or a mixture of both. Consequently, Zym-containing membranes have smaller ability to solubilize phytoceramide gel domains and to regulate membrane passive permeability. Our results strongly support that l_o phase formation is a convergent evolutionary trait in fungi and mammalian cellular membranes.

P-539

Using a self-assembly model to study the impact of genotype-phenotype maps on evolutionary outcomes

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Even with a short genome length and few letters in its genome alphabet, the size of the genotype configuration space can be astronomical, precluding most analytic explanations for the properties of evolution. Using a simple self-assembly model (similar to the polyomino Tile Assembly Model) can qualitatively recover many of the experimentally observed properties of mutation driven evolution, as well as realistically model real protein complexes like haemoglobin. Combinatorial and graph theoretic techniques can be adapted and extended to use on our model, allowing for robust analysis of the otherwise intractable configuration spaces. In particular, these techniques enable analytic construction of Genotype-Phenotype maps, which allows direct comparisons between properties of these maps and properties of the evolution simulations, like the time taken for a population to adapt to a target phenotype. Many of the phenotype multiplicities have non-trivial dependence on the size of the genomic alphabet, emerging from the subtle combinatorics, which can allow selective targeting of phenotypes. Furthermore, regulation at the transcription level can also be explored, and can be used to quantitatively and qualitatively hint at why genomic regulation is a critical mechanism in evolution.

P-538

Fitness value of noisy sensing

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Sensing environment is the first step of cellular adaptation to changing environment. Because the subsequent decision making by cells depends exclusively on the sensing outcome, the reliability of the sensing is crucial for the performance of the adaptation and the fitness gain obtained by the decision-making.

However, the sensing process is usually accompanied by substantial stochasticity in receptor reactions, which induces a heterogeneity of cellular responses in a population.

In this work, we clarify a fundamental relation between the fidelity of the noisy sensing and fitness gain. By using a path-integral formulation of population dynamics with sensing, we specifically show that the fitness gain by the noisy individual sensing is always greater than the mutual information of the sensing. We also discuss the thermodynamic cost of the sensing with the gain of fitness by the noisy sensing.

P-540

To be or not to be: energetics of life, growth suspension and death

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Centre for Synthetic and Systems Biology, University of Edinburgh, UK

Bacteria can survive a variety of external stresses by entering a state of suspended growth that is commonly referred to as dormancy. Such response has historically been considered a univocal low metabolism-low energy state and a vast array of stressors seem to be avoidable through dormancy. Antibiotics are among the most notable examples of such stressors and tolerant, dormant cells are known as persisters. However, recent experiments show that some persisters might survive antibiotic challenges through mechanisms that are, in contrast, energy-consuming. The findings open up the possibility of several different dormant states with distinct cellular energy levels. To verify such a hypothesis, molecular sensors that can provide information on cellular energetics *in vivo* and at the single cell level are needed. To this end, we have successfully optimized the expression of a previously reported QUEEN ATP sensor and characterized in *E. coli* the newly proposed membrane voltage dye, Thioflavin T. Our results provided insights that can be generalized to other dyes, such as TMRM and DiSC₃(5). Using the sensors, we can shed light on the energetics of dormancy when this is induced by different conditions and signals, such as starvation, quorum sensing, and stress signaling molecules.

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P-541

Behaviour and spatial organisation of individual cells in a mutualistic bacterial communityG. Micali, A. dal Co, M. Ackermann
Eawag, Switzerland

The stability of bacterial communities is essential to guarantee the health of humans, animals and plants. Stability crucially depends on the responses to external fluctuations, as well as on the responses to changes in the interactions between the individuals that constitute the community. Previous studies in molecular microbiology often focused on the responses of clonal populations to external perturbations, thus not addressing interactions between species. On the other hand, experiments in microbial ecology are often performed with complex communities without control of the type of interactions involved. Here, we propose an alternative approach. We study a simple engineered community of two species in controlled environments with a defined interaction network. Combining experiments focused on single-cells and community behaviour with theoretical modelling, we study how the behaviour of the community depends on the spatial organization of the individual cells within the community, and how the spatial organization in turn emerge from the interactions between cells.

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Collective behaviour of *e. coli* in complex topologiesR. J. Morris¹, P. Trung^{1,2}, M. Black^{1,2}, K.-C. Lin^{1,2},
Y. Kevrekidis^{1,2}, J. Bos^{1,2}, R. H. Austin^{1,2}
¹University of Edinburgh, UK; ²Princeton University, USA

Bacteria utilize collective behavior to solve problems posed to them by their environment, biofilm formation being a well-known example. In this work we have constructed two classes of spatially complex microenvironments to study how *Escherichia coli* negotiate physically heterogeneous habitats. First, we constructed a microfabricated circular corral for bacteria made of rings of concentric funnels which channel motile bacteria outwards via non-hydrodynamic interactions with the funnel walls. Initially bacteria rapidly move out to the periphery of the corral. Here, fresh nutrients diffuse into the system and the bacteria increase their cell density. After a period of time they are then able to defeat the physical constraints of the funnels by launching back-propagating collective waves. Second, we constructed mazes and other complex topologies which bacteria must solve in order to find new sources of nutrients. Again we found that *E. coli* utilize collective waves to solve the mazes and correctly locate the nutrient sources. Such behavior could provide new insights into how bacteria use collective dynamics to locate nutrients in natural environments and habitats.

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Collective behaviour of *escherichia coli* in spatially complex microenvironmentsR. J. Morris¹, T. Phan², M. Black², K.-C. Lin²,
Y. Kevrekidis², J. Bos², R. H. Austin²
¹University of Edinburgh, UK; ²Princeton University, USA

Bacteria utilize collective behavior to solve problems posed to them by their environment, biofilm formation being a well-known example. In this work we have constructed two classes of spatially complex microenvironments to study how *Escherichia coli* negotiate physically heterogeneous habitats. First, we constructed a microfabricated circular corral for bacteria made of rings of concentric funnels which channel motile bacteria outwards via non-hydrodynamic interactions with the funnel walls. Initially bacteria rapidly move out to the periphery of the corral. Here, fresh nutrients diffuse into the system and the bacteria increase their cell density. After a period of time they are then able to defeat the physical constraints of the funnels by launching back-propagating collective waves. Second, we constructed mazes and other complex topologies which bacteria must solve in order to find new sources of nutrients. Again we found that *E. coli* utilize collective waves to solve the mazes and correctly locate the nutrient sources. Such behavior could provide new insights into how bacteria use collective dynamics to locate nutrients in natural environments and habitats.

P-544

Demographic-noise-induced fixation in subdivided populations with migrationJ. M. Park
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We investigate the stochastic dynamics of a model which consists of subdivided populations of individuals confined to a set of islands. In subdivided populations, migration acts with selection and genetic drift to determine the evolutionary dynamics. The individuals are assumed to be haploid with two types. They reproduce according to their fitness values, die at random, and migrate between the islands. The evolutionary dynamics of a model is formulated in terms of a master equation and is approximated as the multidimensional Fokker-Planck equation (FPE) and the coupled non-linear stochastic differential equations (SDEs) with multiplicative noise. We first analyze the deterministic part of the SDEs to obtain the fixed points and determine the stability of each fixed point. We find that there is a continuous phase transition in the population distribution when the migration rate equals the selection strength in the antisymmetric selection scheme. Next, we derive a quasi-stationary distribution of the multidimensional FPE and predict the fixation probabilities to absorbing states. We also carry out numerical simulations in the form of the Gillespie algorithm and find the analytic results agree very well with the results of simulations.

Posters**– 15. Evolution, ecology, collective and emergent behaviour –****P-545****Phase transition in random adaptive walks on correlated fitness landscapes**S.-C. Park¹, I. G. Szendro², J. Neidhart², J. Krug²¹The Catholic University of Korea, South Korea; ²University of Cologne, Germany

We study biological evolution on a random fitness landscape where correlations are introduced through a linear fitness gradient of strength c . When selection is strong and mutations rare the dynamics is a directed uphill walk that terminates at a local fitness maximum. We analytically calculate the dependence of the walk length on the genome size L . When the distribution of the random fitness component has an exponential tail, we find a phase transition of the walk length D between a phase at small c , where walks are short ($D \sim \ln L$), and a phase at large c , where walks are long ($D \sim L$). For all other distributions only a single phase exists for any $c > 0$. The considered process is equivalent to a zero temperature Metropolis dynamics for the random energy model in an external magnetic field, thus also providing insight into the aging dynamics of spin glasses.

P-547**Low frequency circadian clocks entrain the rest of the clock population in plant leaves**A. Pratap^{1,2}, S. Hodge², A. J. Millar²¹University of Bristol, UK; ²University of Edinburgh, UK

A remarkable variety of biological oscillatory systems undergo collective synchronisation and display a range of spatiotemporal patterns even when there are large variations in the natural frequencies of the population. In plants, circadian clock in a cell comprises of interacting transcriptional feedback loops giving rise to ~ 24 hr oscillatory gene expression even without the exposure to the day/night light cycle (Pokhilko et al. 2012). Using the luciferase reporter gene, we imaged circadian oscillations in the leaves of *Arabidopsis thaliana* plants exposed to constant light for 4 days. Spatiotemporal quasi-regular travelling waves were visible in all of the leaves that were monitored, indicating intercellular coupling (Wenden et al. 2012). We discover that a particular type of the intercellular interaction in concert with high variance in the frequency distribution gives rise to travelling waves. Furthermore, we incorporate our experimental observations into a mathematical model which approximates the leaf cells as a 2-dimensional lattice of weakly coupled limit-cycle oscillators. We use this model to describe the intercellular coupling between circadian clocks in leaves and to explain a scaling relation between the frequency variance and the travelling wavelength.

P-546**Analyzing cross species transformation in laboratory evolution experiments**J. J. Power¹, M. Yüksel¹, V. Kovacova², A. Beyer², B. Maier¹¹Physics Department, University of Cologne, Germany;²CECAD, University of Cologne, Germany

Many bacterial species exchange genetic information by becoming competent for transformation. While the particulars of how each species regulates competence is known, it remains unclear to what extent competence for transformation plays a role in evolution. We have recently quantified the cost of competence under benign conditions in *Bacillus subtilis* and found that even during the stationary state, where *B. subtilis* develops competence, the cost of competence is high. To better understand how competence is maintained despite its cost, we have set up laboratory evolution experiments with the aim of testing for putative benefits of competence. Through sequence divergence, fitness, and growth rates, we can measure the effect competence has on evolution in the presence of UV irradiation and foreign DNA. In preliminary experiments, we found the evolved strain's fitness during exponential growth was considerably higher when bacteria evolved in the presence of ancestral-chromosomal DNA or DNA from a closely related species. The density of single-nucleotide polymorphisms (SNPs) was considerably reduced in the strain evolved in the presence of ancestral-chromosomal DNA. Overall, our techniques will allow us to analyze cross species transformation and calculate a time resolved distribution of fitness effects.

P-548**Enhanced efflux activity facilitates drug tolerance in dormant bacterial cells**

Y. Pu, Z. Zhao, Y. Li, X. S. Xie, F. Bai

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Natural variations in gene expression provide a mechanism for multiple phenotypes to arise in an isogenic bacterial population. In particular, a sub-group termed persisters show high tolerance to antibiotics. Previously, their formation has been attributed to cell dormancy. Here we demonstrate that bacterial persisters, under β -lactam antibiotic treatment, show less cytoplasmic drug accumulation as a result of enhanced efflux activity. Consistently, a number of multi-drug efflux genes, particularly the central component TolC, show higher expression in persisters. Time-lapse imaging and mutagenesis studies further establish a positive correlation between tolC expression and bacterial persistence. The key role of efflux systems, among multiple biological pathways involved in persister formation, indicates that persisters implement a positive defense against antibiotics prior to a passive defense via dormancy. Finally, efflux inhibitors and antibiotics together effectively attenuate persister formation, suggesting a combination strategy to target drug tolerance.

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P-549

Prebiotic capsule formation from thermal heterocomplex molecules of amino acids

S. Sakurazawa, I. Shunsuke

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All existing living organisms have each compartment boundary individually. In material evolution on prebiotic earth, first creature might have been small compartment made from primitive organic materials. All existing cells are compartmented by lipid bilayer membranes. However, lipid molecules are very hard to synthesize prebiotically. Here thermal heterocomplex molecules of amino acids are rise an alternative possibility as prebiotic boundary of cells. Thermal heterocomplex molecules of amino acids form micro spherical particles of a few micrometer diameter. These particles dissolve into aqueous solution due to environmental changes such as temperature, pH and polarity of the solution. While these particles dissolve into their environmental solution, capsule like structures formed around the dissolving particle in the flow of thermal heterocomplex molecules dissolved from interface of the particle under the non-equilibrium environment. These capsules structures have nano-sized pores since these capsules are formed with nano-sized particles forming nano-sized pore among these particles. Therefore, these structures encapsulate macromolecules while exchanging small molecules. It suggest that such a structure might have been possible prebiotic boundaries.

P-550

Trace element distribution in feathers of *Pygoscelis papua*J. S. Souza¹, C. C. Silva², E. S. Costa³, A. Covaci⁴, J. P. M. Torres¹

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Seabirds are sentinels of the impact of contaminants on marine ecosystems. Feathers are a non-destructive biomonitoring tool of trace elements and act as archives of the animal's exposure over time. The studied species, *Pygoscelis papua*, is restricted to regions close to the Antarctic continent, being thus used as a sentinel to provide data on local contamination. This study aimed to investigate the elemental composition of *P. papua* feathers by X-ray fluorescence (XRF). Fieldwork was carried out during the summer of 2013/2014 at the Hennequin Point, King George Island, Antarctica. The animals were captured and breast feathers were cut (n=14). During the analytical procedure, we detected 17 trace elements, including toxic trace elements and major essential elements. The elements Al, Ca, Cl, Fe, K, Mg, Na, P, and Si were detected in all samples, while the elements Ag, Br, Cr, Cu, F, S, Ti and Zn were detected only in few samples. The highest and lowest concentration detected was for F and Br. This is the first application of a non-destructive efficient and useful technique (XRF) to quantification of trace elements in penguin feathers. This study provides data for the further understanding of the uptake and mobilization of chemical elements in feathers.

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P-551 (O-96)

Coupling fluorescence microscopy and electrochemistry to investigate single mitochondria metabolismV. S. Vajrала¹, F. Sekli Belaïdi², G. Lemercier², D. Zigah¹, J. Launay², P. Temple-Boyer², N. Sojic¹, S. Arbault¹¹University of Bordeaux, CNRS, INP-Bordeaux, France;²LAAS-CNRS, Université de Toulouse, France

Mitochondria are major cell organelles as being the main source of ATP through the oxidative phosphorylation. They also play major roles into other metabolic pathways (Krebs cycle, lipid synthesis, calcium, redox signalling) and when defective, they are involved into severe pathologies (myopathies, neurological disorders...). Consequently, new methodological approaches are required to decipher mitochondrial activities and provide tools for diagnosis. We have developed microsystems, ElecWell platforms, which combine electrochemical and optical sensing abilities. These are based on the integration of platinum nanoelectrodes (RNE, 200 nm thickness) into SiO₂-based microwell arrays (10² to 10⁶ wells; vol. < 1 pL). RNE exhibit high current density, fast response time, and high S/N ratio. The glass substrate of microsystems allows the observation by microscopy within all wells. A suspension of mitochondria is let to sediment on the array; mitochondrial trapping in each well is monitored by fluorescence, owing to their NADH or membrane potential. Simultaneously, we monitor electrochemically their oxygen consumption rate in response to activators and inhibitors of the respiratory chain. ElecWells offer unprecedented resolution to assess single mitochondria activities and dysfunctions.

P-553 (O-98)

Protons at the membrane water interface

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Proton diffusion along biological membranes is vitally important for cellular energetics. It occurs almost as fast as that in pure water (Serowy *et al.* 2003. *Biophys. J* 84:1031). Protons migrate without significant contributions from jumps between ionizable groups on the membrane surface (Springer *et al.* 2011. *PNAS* 108:14461). The process requires nothing but structured water at the boundary of a hydrophobic phase (Zhang *et al.* 2012 *PNAS* 109:9744). So far the origin of the Gibbs activation energy barrier ΔG^\ddagger_r remained enigmatic that opposes proton surface-to-bulk release. Here we determined ΔG^\ddagger_r from Arrhenius plots of (i) protons' surface diffusion constant and (ii) surface-to-bulk release rates. We found both parameters by (α) photo-releasing protons from a membrane patch at different temperatures and (β) monitoring their arrival at a distant patch. The results disproved that quasi-equilibrium exists between protons in the near-membrane layers and in the aqueous bulk. Instead, non-equilibrium kinetics is consistent with this experiment. ΔG^\ddagger_r only contains a minor enthalpic contribution that roughly corresponds to the breakage of a single hydrogen bond. This work reconciles the delayed proton surface-to-bulk release with protons weak bonding to surface water molecules.

P-552 (O-97)

Retinal thermal equilibrium, photocycle and energy conversion in the microbial seven-transmembrane photoreceptorsX.-Y. Ding^{1,2}, C. Sun¹, H.-L. Cui¹, Y.-J. Gao¹, J. Wang¹, Y.-N. Yang¹, A. Watts³, X. Zhao¹¹East China Normal University, China; ²Penn State College of Medicine, USA; ³University of Oxford, UK

Light-driven proton pumps, a number of the microbial rhodopsin family seven-transmembrane receptors, distribute widely among Archaea, Eubacteria, and Eukaryota for harvesting and converting light energy in a wider spectral range. The common feature of those proteins is that the retinal chromophore maintains a *cis-trans* thermal equilibrium in the dark-adapted state. Absorption of a photon causes photoisomerization of the chromophore from the all-*trans* to the 13-*cis*, 15-*anti* configuration that triggers a series of structural rearrangements in the protein and initiates the vectorial translocation of a proton out of the cell. However, little is to know about how the retinal *cis-trans* thermal equilibrium affects the proton translocation mechanism and further impacts on the energy conversion rate. 2D solid-state NMR of specifically labelled receptors, reinforced with molecular dynamic simulations, mutational analysis and functional assays, supported by and compared with rigid-atom crystal structural models are employed to address this challenge. Shifting of the retinal *cis-trans* thermal equilibrium to an either *cis* or *trans* dominated state will affect the proton photocycle mechanism and further decrease the energy conversion rate.

P-554

Redox-dependent proton translocation in *cbb3* oxidaseC. A. Carvalheda^{1,2}, A. V. Pisliakov^{1,2}¹School of Life Sciences, University of Dundee, UK; ²School of Science and Engineering, University of Dundee, UK

Heme-copper oxidases (HCOs) couple the chemical energy released in reduction of oxygen to water, the final step of the respiratory chain, to the active proton translocation across the membrane, thus contributing to the establishment of a proton gradient, which is essential for ATP synthesis. *Cbb3* (or C-type) cytochrome *c* oxidases are a highly divergent group and the least studied members of the HCO superfamily. They exhibit unique structural and functional features, and have an essential role in metabolism of clinically relevant human pathogens. The functioning mechanism of *cbb3* oxidases, namely the proton transfer/pumping mechanism via a single proton channel, is still poorly understood.

In this work we use a combination of computational tools to get atomic-level insights into the water dynamics and proton translocation in *cbb3* oxidase. Recently we described the proton transfer pathways for the “chemical” and “pumped” protons, and proposed a redox-driven pumping mechanism (Carvalheda 2017 *BBA-Bioenerg.* 1858 396). Here we report the impact of redox changes in the binuclear centre on the proton pathways and protonation equilibria of key residues. Our results contribute to a better understanding of *cbb3* mechanism and provide ideas for further experimental and computational studies.

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– 16. Molecular and cellular processes of energy transduction –

P-555

In situ solid-state NMR study of a new photoreceptor with two chromophoresX.-Y. Ding^{1,2}, C. Sun¹, Y.-J. Gao¹, H.-L. Cui¹, J. Wang¹, Y.-N. Yang¹, F. Tian², X. Zhao¹¹East China Normal University, China; ²Penn State College of Medicine, USA

Archaeorhodopsin-4 (aR4) is an unknown structured membrane protein from *Halobacterium species* XZ515 found in a salt lake in Tibet, China. It has a 7-transmembrane topology and functions as a proton pump similar to that of bacteriorhodopsin (bR). The retinal chromophore is covalently bound to the lysine 217 on the helix G through a protonated Schiff base. Absorption of a photon causes photo isomerization of the chromophore from the all-trans to the 13-cis, 15-anti configuration and triggers a series of structural rearrangements of the protein that initiates a vectorial translocation of a proton out of the cell. There are two major differences between aR4 and bR. First, aR4 has an opposite temporal order of proton uptake and release at neutral pH. Second, aR4 has not only a retinal as the premier chromophore, but also a second chromophore bacterioruberin. In order to address these questions, a novel heterogeneous expression system is established to express the protein with two chromophores. The photocycle, proton translocation mechanism, function of the second chromophore, and energy conversion efficiency are studied through 2D solid-state NMR of specifically labelled receptors, reinforced with molecular dynamic simulations, mutational analysis and functional assays.

P-557

First Principles Design of Organic Piezoelectric DevicesS. Guerin^{1,2}, A. Stapleton^{1,2}, D. Chovan^{1,2}, S. A. Tofail^{1,2}, D. Thompson^{1,2}¹Department of Physics, University of Limerick, Ireland;²Bernal Institute, University of Limerick, Ireland

Piezoelectric materials exhibit the unique characteristic of becoming electrically charged when strained and conversely, becoming deformed in the presence of an electric field. Inorganic piezoelectric materials have been exploited for decades as nanogenerators, biosensors, resonators, acoustics, and in scanning probe microscopy (SPM). Here we present organic crystals as a basis for such applications, based on experimentally validated quantum mechanical models. Our models can quantify the piezoelectric response of small biomolecular crystals, and uncover significant electromechanical coupling along hidden crystallographic planes. In this project we present both our theoretical and experimental data on amino acid crystals, highlighting their low permittivity and high flexibility. Amino acids are the building blocks of proteins and other biological structures and in their uncrystallised form regulate a number of our bodies' functions. Here we discuss the future that crystallised biomolecules could have in energy harvesting and sensing technologies, as well as the implications of discovering piezoelectricity at the foundations of our biology.

P-556

Hydrogen cycle in purple non-sulfur bacteria: relationship between nitrogenase and hydrogenaseL. Gabrielyan, H. Sargsyan, L. Hakobyan, A. Trchounian
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Purple non-sulfur bacteria such as *Rhodobacter sphaeroides* from Armenian mineral springs can produce bio-hydrogen (H₂) during photo-fermentation of various organic acids. *R. sphaeroides* contains [Mo-Fe]-nitrogenase, which catalyzes the conversion of H⁺ to H₂ in nitrogen-limited conditions. This process requires large amount of ATP formed during photosynthesis. *R. sphaeroides* also contains [Ni-Fe]-hydrogenase, which can “recycle” a considerable portion of H₂ generated by nitrogenase. Both enzymes catalyze the redox reaction: 2H⁺ + 2e⁻ → H₂, and thereby form the hydrogen cycle through the bacterial membrane. Bacterial anaerobic growth is coupled with decrease of redox potential from positive to negative value, which describes transfer of electrons and proton motive force formation. During photo-fermentation of succinate, malate and acetate the redox potential of bacterial growth medium decreased to negative values (-450 - -600 mV), which is correlated to H₂ generation, because the standard redox potential of the 2H⁺/H₂ couple is -414 mV, and the reduction of H⁺ to H₂ is observed under reducing conditions. Redox potential of bacteria depends on the reduced and oxidized products of photo-fermentation, as well as on pH, and might have a role in hydrogen cycle regulation.

P-558

Magnetic-isotope effects of Mg-25 and Zn-67 in ATP-hydrolysis driven by myosinV. K. Koltover¹, R. D. Labyntseva², S. O. Kosterin²¹Institute of Problems of Chemical Physics, RAS, Chernogolovka, Moscow Region, Russian Federation;²Palladin Institute of Biochemistry, NAS, Kiev, Ukraine

Among three stable isotopes of magnesium, ²⁴Mg, ²⁵Mg, and ²⁶Mg with natural abundance 76.7, 10.1, and 11.2 %, only ²⁵Mg is magnetic (nuclear spin $I = 5/2$) while ²⁴Mg and ²⁶Mg are nonmagnetic ($I = 0$). In the experiments with myosin isolated from uterus muscle we have revealed that the rate of the enzymatic ATP hydrolysis is 2.0–2.5 times higher with ²⁵Mg, than the rates with nonmagnetic ²⁴Mg or ²⁶Mg or with natural MgCl₂. The similar magnetic isotope effect was revealed with zinc. Among five stable isotopes, ⁶⁴Zn, ⁶⁶Zn, ⁶⁷Zn, ⁶⁸Zn and ⁷⁰Zn with natural abundance 48.6, 27.9, 4.1, 18.8 and 0.6 %, only ⁶⁷Zn is magnetic ($I = 5/2$). While Zn²⁺ performs the cofactor function less efficiently, than Mg²⁺, it was found that the rate of the enzyme ATP hydrolysis with ⁶⁷Zn is 40–50 % higher as compared to the nonmagnetic ⁶⁴Zn or ⁶⁸Zn. Thus, there is a rate-limiting step (“bottle-neck”) in ATP hydrolysis catalyzed by myosin that is accelerated by the magnetic fields of the nuclear spins of ²⁵Mg and ⁶⁷Zn. As the most plausible physical mechanism of the effect, we assume that the nuclear spin promotes the conformation-deformation excitation of the enzyme macromolecule into the relevant triplet excited state (coherent bosons or soliton), thereby accelerating the chemomechanical cycle of the enzyme.

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P-559

Modelling the stability of oscillations in altered metabolic statesG. Lancaster¹, Y. F. Suprunenko², H. Perkins¹, A. Stefanovska¹¹Lancaster University, UK; ²University of Liverpool, UK

Biological oscillations are found on many scales, one of the smallest being cellular energy metabolism. The dynamics of metabolic oscillations varies depending on the state of the cell. Understanding the origin of these variations may allow the identification of altered metabolic states, for example the Warburg effect observed in cancer cells.

The recent theory of chronotaxic systems [1] provides a theoretical framework in which systems can have stable yet time-varying dynamics, thus seemingly random biological systems can in fact be deterministic. Based on this principle, a model of cellular energy metabolism was developed to capture the main behaviour observed in metabolic oscillations, and show how chronotaxicity changes in the system could be used to track metabolic transitions [2].

Recent extensions to the model take a more realistic approach to glycolytic oscillations, demonstrating how their widely observed dynamics may result from ensembles of many coupled oscillators. Here, we discuss the model, and demonstrate how chronotaxicity can be observed in real experimental data recorded in yeast cells [3].

[1] Suprunenko *et al.*, Phys. Rev. Lett., 111(2), 024101, 2013

[2] Lancaster *et al.*, Sci. Rep., 6:29584, 2016

[3] Gustavsson *et al.*, FEBS J. 279(16), 2837–2847, 2012

P-561

Organic molecules-driven energy conversion of photoluminescence from single-walled carbon nanotubes

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In terms of superior optical properties of carbon nanotubes (CNTs), they can be considered as new materials for energy conversion. Previous energy conversion system of CNTs uses organic molecules, particularly fluorescent dye-labeled DNA, because the system utilize energy transfer. In this study, we tried to control the photoluminescence (PL) from CNTs by regulating base-sequences of DNA without fluorescence dyes. The hybrids of 30-mers of thymine (T30) or cytosine (C30) and CNTs (T30–CNTs and C30–CNTs, respectively) were reacted with DNA. As the base-sequences of DNA, we adopted T30, A30, C30 and G30. For comparison, we prepared CNT solution which were dispersed with double-stranded DNA(dsDNA).

PL spectra from T30–CNTs with T30 and A30 were obtained at 1122nm and 1130nm, respectively. The difference in the peak energy between T30–CNTs with T30 and A30, 8meV, suggests that the A30 molecules hybridized with T30 molecules on CNT surfaces. Furthermore, the peak energy from T30–CNTs with A30 was corresponded with that from dsDNA-CNTs. On the contrary, there were no energy conversion between C30–CNTs with C30 and A30. Our results show that CNTs have promising potentials for applications such as photoelectric conversion or optical switch devices which are driven by organic molecules.

P-560

SERS-based study of cytochrome c properties in heart mitochondria from health and diseased animalsE. I. Nikelshparg¹, N. A. Brazhe¹, A. A. Bayzhumanov¹, L. I. Deev¹, E. A. Goodilin², A. A. Semenova², A. S. Sarycheva², O. Sosnovtseva³, G. V. Maksimov¹¹Faculty of Biology, Lomonosov Moscow State University, Russian Federation; ²Faculty of Material Sciences, Lomonosov Moscow State University, Russian Federation;³Faculty of Medical and Health Sciences, University of Copenhagen, Denmark

Alterations in cytochrome (cyt) properties in mitochondria (MC) contribute to ROS production and apoptosis initiation which implicates MC in several cardiovascular diseases. We demonstrated that surface-enhanced Raman spectroscopy (SERS) provides information about the redox state and conformation of cyt c in intact functional MC placed on plasmonic silver nanostructures. Using 514nm and 532nm lasers we demonstrated that MC SERS spectra tell about redox state and conformation of cyt c heme and depend on respiration rate (RR), uncoupling and inhibition of ATP synthesis; the decrease in the intermembrane space by hypotonic medium or valinomycin increase overall SERS intensity and leads to appearance of cyt b peaks. SERS spectra from MC isolated from spontaneously hypertensive rat hearts differ from normotensive Wistar Kyoto rat hearts under modulation of MC activity. All results are in a good agreement with RR and rates of ATP production by MC. NAB acknowledges financial support from RFBR and Moscow Government (grant 15-34-70028-mol-a-mos).

P-562

Long term impairment of cognitive functions and alterations of NMDAR subunits after continuous microwave exposure

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The long term effects of continuous microwave exposure can not be ignored for the simulation of the real environment and increasing concerns about the cognitive mal-effects of microwave exposure. In this study, 220 male Wistar rats were exposed by a 2.856 GHz radiation source with the average power density of 0, 2.5, 5 and 10 mW/cm² for 6 min/d, 5d/w and up to 6 weeks. The MWM task, EEG analysis, hippocampus structure observation and the western blot were applied until the 12m after microwave exposure to detect the spatial learning and memory abilities, the cortical electrical activity, changes of hippocampal structure and the NMDAR subunits expressions. The results found the continuous microwave exposure could cause the dose-dependent long term impairment of spatial learning and memory, the abnormalities of EEG and the hippocampal structure injuries. The decrease of NMDAR key subunits and the phosphorylation might contribute to the cognitive impairment.

Keywords: continuous microwave exposure, rats, cognitive functions, hippocampus, NMDAR, long term impairment.

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Posters

– 16. Molecular and cellular processes of energy transduction –

P-563

Singlet oxygen production in intact microalgae and cyanobacteria: mechanism and detection methods

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The production of singlet oxygen ($^1\text{O}_2$) is important in cellular processes like cellular signaling, defense and it is associated with damage to cellular components such as proteins, lipids and nucleic acids. In photosynthetic systems, it is produced via interaction of molecular O_2 with ^3Chl s in the PSII reaction center, as well as in the light harvesting antenna. Although various methods such as luminescence at 1,270 nm, fluorescent probes method and electron paramagnetic resonance are available for $^1\text{O}_2$ detection in isolated photosynthetic systems, these sensor molecules are unable to penetrate the cell wall of intact cyanobacteria and microalgae, which seriously limits the possibility to study the role of $^1\text{O}_2$ in vivo. In order to overcome this difficulty, we have developed a method which is based on chemical trapping of $^1\text{O}_2$ by histidine. Here, an overview will be given on previously available $^1\text{O}_2$ detection methods and their limitations compared to our established method. Extra- and intracellular production of $^1\text{O}_2$ in intact *Symbiodinium* cells will also be reported where we show that $^1\text{O}_2$ induced inactivation of *Symbiodinium* cells, may be involved in triggering the expulsion of *Symbiodinium* cells from the coral host, which leads to coral bleaching.

P-565

Studies of transmembrane LH complexes in a natural-like environment at the single-liposome levelM. Tutkus², P. Akhtar¹, G. Trinkūnas², D. Rutkauskas², P. Lambrev¹

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Photosynthetic organisms during photosynthesis by utilizing light energy, from non-organic compounds are producing various organic compounds. Initial absorbed light energy transfer processes in plants are well adapted to work in naturally varying light intensity. One aspect of this flexibility is called non-photochemical fluorescence quenching (NPQ) of light harvesting complexes (LHC). In plants, the major player of the NPQ is LHCII and here we are investigating its role on the properties of NPQ. For these studies, we employ a new synergy in this research field between single-molecule fluorescence microscopy methods and strategy of transmembrane protein reconstitution into liposomes. This combined method allows us to circumvent negative surface effects and study truly liposome reconstituted LHCII molecules. Our assay consists of thylakoid lipid mixture liposomes containing biotinylated lipids that allow us to anchor liposomes onto PEGylated glass surface via Neutravidin and to keep them intact for long periods of time. Liposomes also contain lipid-dyes that by the use of co-localisation helps to identify successfully reconstituted LHCII. Lipid-dyes allow us to estimate liposome sizes and therefore access membrane curvature sensing of the LHCII and its on NPQ.

P-564

The influence of cell permeability and membrane voltage on loading of membrane voltage dyesG. P. Terradot^{1,2}, L. Mancini¹, P. S. Swain², T. Pilizota¹

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Maintenance of membrane potential and Proton Motive Force (PMF) are critical for cell growth and survival. For example, PMF, through respiration, allows production of the energy currency of living cells, ATP, and powers the transport of many substrates such as amino acids.

Several experimental methods that attempt to measure membrane voltage rely on the use of (semi)-permeable charged fluorescent molecules (dyes) such as Thioflavin T (ThT) and Tetramethylrhodamine, Methyl Ester, Perchlorate (TMRM). Their steady-state intracellular concentration depends on the electrochemical potential of the cell, which is a function of the membrane potential and the gradient of the dye across the membrane.

Our experimental data and mathematical model show that the expected relationship between the intracellular concentration of the dye and the membrane voltage depend on the growth conditions. For instance, extracellular pH, carbon source present in the media or cell permeability significantly change the membrane potential given a certain intracellular concentration of the dye.

Our results emphasize the need for precise calibration of the dye-based membrane voltage measurements for each specific condition in which the experiments are performed.

P-566

Mitochondrial membrane potential positively regulates vascular smooth muscle cell contraction

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Exposure of the vasculature to metabolic disturbances leaves a persistent imprint on smooth muscle cells (VSMCs). However, the effects of metabolic influx on VSMCs function and the underlying mechanism are poorly understood. Here, we found that metabolic stimulation induces VSMCs contraction through enhancement of mitochondrial membrane potential ($\Delta\Psi_m$). Glucose challenge induced vascular contraction and elevation of blood pressure in humans. Such contraction takes effects in VSMCs, and is dependent on mitochondrial oxidation but not mitochondrial ATP formation. Using inophores (FCCP, nigericin, valinomycin and monensin) revealed that glucose-induced vascular contraction was mediated by enhancement of $\Delta\Psi_m$. Inhibition of $\Delta\Psi_m$ elevation suppressed vascular contraction following glucose challenge. Mechanistically, $\Delta\Psi_m$ regulates GTPase activity of miro (mitochondrial Rho). An increase of $\Delta\Psi_m$ activated miro which stimulated Rho kinase activity, resulting in myosin light chain phosphorylation. Furthermore, we observed that the homeostasis of $\Delta\Psi_m$ in response to metabolic stimulation was disrupted in diabetic mice, which resulted in a sustained elevation of blood pressure. These results suggested that $\Delta\Psi_m$ positively induces VSMCs contraction through activation of miro.

Posters

– 17. Membrane permeation: channels –

P-567 (O-63)

Quantum calculations on the voltage sensing domain (VSD) of the Kv1.2 potassium channelA. M. Kariev, M. E. Green
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A 976 atom subset of the Kv1.2 channel voltage sensing domain (with water) was optimized at Hartree-Fock level. Side chains pointing away from the center of the VSD were truncated; S1,S2,S3 end atoms were fixed; S4 end atoms were fixed in some calculations, free in others. Following optimization, single point calculations (B3LYP/6-31G**) allowed accurate comparisons of energies. Open conformations (*i.e.*, membrane potentials ≥ 0) are consistent with the known X-ray structure of the open state when some salt bridges in the VSD are not ionized (H^+ on the acid, S4 end atoms fixed or free). The backbone of the S4 segment, free or not, moves no more than 2.5 Å upon switching from positive to negative membrane potential, and the movement is wrong for closing the channel. This leaves H^+ motion as gating current. Groups of 3-5 side chains are important for proton transport, based on the calculations. A pair of steps: proton transfer from a tyrosine, Y266, through arginine (R300), to a glutamate (E183), is a key result, providing approximately 25% of gating current. Calculated charges on each arginine and glutamate are appreciably less than one. The calculation predicts that a Y266F mutation would have a drastic effect on the channel, possibly killing it. Alternate interpretations of experiments usually understood in terms of the standard model are shown to be plausible.

P-569

Tethered phospholipid bilayers for picomolar detection of cholesterol-dependent cytolysinsT. Penkauskas, M. Jankunec, G. Valincius
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Formation of plasma membrane is considered as a crucial event during evolution and life, as known today, would not be possible without them. Actual plasma membranes contain a complex, heterogeneous distribution of lipids and membrane proteins which interact to create important biological functions. To investigate this complex membrane environment significant progress has been made to model native membranes. The most common systems include lipid monolayers, lipid vesicles, and supported lipid bilayers (SLBs). Particular group of solid supported bilayers, tethered bilayers (tBLMs), are considered as perspective experimental platforms for membrane biosensors. In particular, the modulation of the ionic conductance of tBLMs may be utilized to develop biosensors of the membrane damaging agents such as pore-forming toxins.

We have used the tBLM platform for the detection and visualization of Cholesterol-dependent Cytolysins (CDCs), using electrochemical impedance spectroscopy (EIS) and atomic force microscopy (AFM) techniques. tBLM modification in aqueous environment has been performed, using Methyl- β -cyclodextrin (MBCD) and cholesterol complex, to achieve picomolar CDC sensitivity.

P-568 (O-102)

Mechanism of loop C closure in the glycine receptor and its relevance for partial agonismM. A. Dämgen¹, T. Greiner², R. Lape², L. G. Sivilotti²,
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Cys-loop receptors mediate fast synaptic transmission and are pivotal drug targets. Agonist binding opens the transmembrane pore, allowing ions to flow into the cell. Previous research has identified agonists with varying ability to open the ion channel, yet an explanation of partial agonism at atomistic resolution remains elusive. An understanding would be crucial for drug design, since depending on the clinical situation, the ideal therapeutic drug should elicit a fine-tuned ion flow that is somewhere in between that of a full agonist and a silent antagonist. We performed molecular dynamics simulations of the glycine receptor with the full and partial agonists in the orthosteric binding site. For the first time, we report a detailed atomistic mechanism of loop C closure, which is the first step in the signal-transduction mechanism. Our findings suggest that agonist efficacy is linked to the ability of stabilising loop C in a closed conformation. Moreover, we find that a stable water molecule in the binding pocket plays a crucial role for all examined ligands. The observed glycine binding mode is in excellent agreement with a recent crystal structure where density of glycine with a stable water molecule in the binding pocket is discernible.

P-570 (O-103)

The Structure of an Open Activated Sodium Channel Reveals the Molecular Basis of Gating and DiseaseA. Sula, J. Booker, B. A. Wallace
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Voltage-gated sodium channels (Navs) play essential roles in excitable tissues, with the activation and opening of these channels resulting in the initial phase of the action potential. The cycling of Navs through closed and inactivated states and the related opening/ closing of other ion channels lead to exquisite control of intracellular ion concentrations in both prokaryotes and eukaryotes.

Our new high resolution crystal structure of the NavMs prokaryotic sodium channel (Sula et al, 2017), provides the only view to date of an open activated channel. It shows the interactions of the voltage sensor, S4-S5 linker, pore, and C-terminal domains (CTDs). The S4 helix is in an activated conformation and the pore gate is open at the intracellular surface. The CTD is coiled-coil that acts as a “glue” between the monomers that comprise the tetrameric channel. The conformation includes a heretofore unseen extensively hydrogen-bonded and salt-bridged interaction motif involving the sodium-channel specific S3 Trp, the S4-S5 linker, the end of the S6 transmembrane region and the top of the CTD. This structure provides the basis for understanding the processes of gating and ion translocation in Navs, and a novel insight into one of the basis of human diseases.

Posters

– 17. Membrane permeation: channels –

P-571 (O-104)

The mechanism of drilling β -barrel pores into lipid membranes by an earthworm protein Lysenin

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Pore-forming proteins (PFPs) are widespread in nature and have important physiological roles in attack and defense mechanisms via formation of pores in lipid membranes of target cells. They are classified as either α - or β -PFPs, based on the secondary structure elements that form the transmembrane part of the pore. Members of aerolysin family form β -barrel shaped pores. The family expands from bacteria to vertebrates, with many bacterial representatives serving as crucial virulence factors. Lysenin is one of the few described eukaryotic members of this family from the earthworm *Eisenia fetida*. It is present in the coelomic fluid of earthworms to act defensively against parasitic microorganisms. It has a high affinity for sphingomyelin in membranes, and can be used as a tool for visualizing distribution and dynamics of sphingomyelin in cells. I will present the crystal structure of the lysenin pore (Podobnik et al., *Nat Comm* 2016), which provides important insights into the mechanism of pore assembly as well as general features of this family of nanopores. Our results are relevant for understanding of pore formation by other aerolysin-like PFPs involved in bacterial pathogenesis, as well as potential application of such pores in medicine and nanobiotechnology.

P-573

Accumulation of cardiac inwardly rectifier potassium channels in a rat model of epilepsy

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The mechanism underlying epilepsy is not fully understood, and the rate of sudden death from cardiovascular complications of epilepsy is approximately 15%. Since the inwardly rectifying potassium channel 4.1(Kir4.1) has been shown to work dysfunctional in epilepsy; it was predicted that the relationship between the brain and the heart could be elucidated through Kir channels which has the role in repolarization phase of cardiac action potential. In line with this objective, chronic epilepsy was induced in animals by administration of 40 mg/kg pentylenetetrazole(PTZ) intraperitoneally every two days to rats for one month. Wistar albino rats (n=40) were divided in 4 groups as female/male control and epilepsy groups. In the heart tissue of the PTZ-induced epileptic rats, the accumulation of Kir channels protein was revealed by Western blot analysis. Real-time polymerase chain reaction analysis also showed that mRNA levels of Kir channels were significantly changed in cardiac tissue of epileptic rats. These results demonstrate the importance of investigating electrophysiological studies and ion channels in the epileptic heart. These findings suggest that cardiac pathology may be present in epileptic patients and that the accumulation of Kir channels may play a role in this pathology.

P-572

Examination of ECG recording and cardiac potassium channels' genes in a mice model of demyelination

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It is known that mortality rates in multiple sclerosis (MS) patients are rising due to cardiovascular diseases. Therefore, it is of great importance to investigate the mechanisms of cardiovascular disease risks such as myocardial infarction, paralysis and atrial contraction that may accompany MS. For this purpose, in order to elucidate cardiac problems in patients with MS; we examined the gene expression patterns of cardiac inwardly rectifier potassium channels (Kir) which play a key role in cardiac excitability by contributing to the repolarization phase of action potential and recently identified as a target of an autoantibody response in patients, in a mice model of demyelination induced by cuprizone. Demyelination was confirmed by immunohistochemistry using an anti-proteolipid protein antibody in the corpus callosum of mice fed with cuprizone. Real-time polymerase chain reaction (RT-PCR) analysis revealed that fold change of Kir channels' genes (KCNJ10 and KCNJ12) are significantly up-regulated in the heart. Besides this, surface electrocardiographic recordings from mice showed that significant changes in RR interval and P wave. This study may give the idea of studying Kir channels in more detail and these channels could have a role in mechanism of cardiac problems in MS.

P-574

ATP consumption and cell viability of breast adenocarcinoma cell lines after electroporation mediated transport of calcium ions

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Electroporation (EP) applied with calcium ions can induce acute ATP depletion due to a combination of increased cellular use of ATP.

Two human adenocarcinoma cell lines were used: MCF-7/WT and MCF-7/DX. The electroporation was performed with the following parameters: 800, 1000, 1200 and 1400 V/cm in combination with calcium ions solution (0.25, 0.5, 1 and 5 mM). There was evaluated cell viability and the ATP consumption of Ca²⁺ delivered into malignant cells via electroporation process. Voltage-dependent calcium ion channels were determined by immunocytochemical ABC method. Moreover a transient receptor potential ion channel (LTRPC2), subtype involving Ca²⁺ influx and replenishment was visualized by immunofluorescent method.

The best anticancer effect was observed after the application of EP+Ca²⁺. The cell survival rate was significantly lower in comparison to cells treated electroporation alone. EP with Ca²⁺ stimulated antitumor response after 48h where ATP consumption increased significantly and the number of viable cells decreased. The satisfactory results were obtained at higher voltages.

The obtained results can be a useful preclinical study for in vivo studies involving application of EP in combination with calcium ions as an efficient anticancer drug in cancer cells.

Posters

– 17. Membrane permeation: channels –

P-575

Investigation of the pore aperture width required for Kir channel gatingK. A. Black^{1,2}, D. M. Miller¹, J. M. Gulbis¹¹Structural Biology Division, The Walter and Eliza Hall Institute of Medical Research, Australia; ²Department of Medical Biology, The University of Melbourne, Australia

Potassium (K^+) channels allow rapid K^+ diffusion across cell membranes. Control over K^+ levels is essential for normal cellular activity, both for basic processes such as maintenance of electrolyte balance, as well as more complex processes such as electrical signalling in neurons. In order to function correctly, channels need to open and close in a controlled manner, a process known as 'gating'. During gating, the channel's pore width is thought to change, with an 'open' channel having a wide pore aperture, and a 'closed' channel a narrow one.

The magnitude of change required during gating of inwardly rectifier K^+ (K_{IR}) channels was evaluated by covalently linking adjacent subunits together. This constrained the movement of the helices delineating the pore and so effectively limited the pore diameter to the narrow conformation. Three crosslinking agents were employed, and formation of crosslinks was assessed by SDS-PAGE, x-ray crystallography and native mass spectrometry. Channel function was assessed by electrophysiology and fluorescent liposome flux assays.

Results indicated that channels with a limited narrow pore aperture were able to function as effectively as wildtype channels, suggesting that in K_{IR} channels, gating is not explained by a conformational change of the pore.

P-577

Characterization of a novel high-selectivity Kv1.3 inhibitor peptide

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Ion channels expressed in T lymphocytes play key roles in the control of the membrane potential and calcium signaling. The physiological function of effector memory T lymphocytes can be modulated selectively by peptide toxins acting on the Kv1.3 K^+ channels. Since Kv1.3 specific peptide toxins are considered to have a significant therapeutic potential in the treatment of autoimmune diseases, the discovery of new toxins is highly motivated. Vm24, isolated from the venom of *Vaejovis mexicanus smithi*, inhibits Kv1.3 with high affinity ($K_d = 2.9\text{pM}$). However, other biologically important channels, such as hKCa3.1, mKv1.1 and hKv1.2 were partially blocked by the peptide at 10nM concentration, whereas other channels tested were unaffected. A novel peptid toxin from the same scorpion named sVmKTx half-blocked the Kv1.3 currents in 770pM concentration. In contrast, we could not observe significant effects of sVmKTx (100nM) on currents of the following ion channels: hKv1.1, hKv1.2, hKv1.4, hKv1.5, rKv2.1, hKCa3.1, hKCa1.1 and hNav1.5. Our experiments confirm a higher selectivity of sVmKTx for Kv1.3 at a cost of a decrease in the Kv1.3 affinity.

P-576

Comfortably numb: determining binding behavior of local anaesthetics using molecular simulations

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Voltage-gated sodium channels are transmembrane proteins responsible for generating action potentials in nerve and muscle cells. Mutations of sodium channels are responsible for many disorders, including epilepsy and chronic pain. Thus, they are key targets for a variety of compounds, including 'local anaesthetics,' which block the central pore of the channel, inhibiting sodium transport. However, local anaesthetics inhibit all nine human sodium channel subtypes, so designing compounds to target specific subtypes would allow for new uses and targeted therapy, reducing side effects. The majority of existing local anaesthetics have protonatable amine groups, which have pKas close to physiological pH, so the compounds exist as both neutral and protonated in the blood. Understanding how each state blocks the pore is important to local anaesthetic action, and informing subtype selectivity. To determine how a range of local anaesthetics interact with sodium channels, we have used molecular dynamics simulations with enhanced sampling. This study proposes binding sites for both charged and neutral compounds, as well as a mechanism of action of compounds with both neutral and charged forms, better informing sub-type selectivity, and opening the door to new uses and targeted therapy.

P-578

An investigation of a role of fixed charge in the selectivity filter of NaChBac

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Selectivity of cation ion channels can be explained in terms of a fixed negative charge (Q_f) associated with the amino acid residues in the selectivity filter (SF) of the channel. A patch clamp investigation of mono- and di-valent cation permeation of NaChBac and its mutants exhibiting different Q_f values (ranging from 0 to -12) confirm the importance of Q_f as a determinant of NaChBac ion selectivity. Specifically, we have shown that (1) deletion or neutralisation of Q_f leads to loss of cation permeation, (2) increasing Q_f permits the permeation of monovalent cations with larger ionic radii (K^+ , Rb^+ and Cs^+) and permeation of divalent cations and (3) channels with $Q_f \geq -8$ exhibit a divalent blockade and anomalous mole fraction effect in solutions containing mixtures of Na^+ and Ca^{2+} . However, NaChBac mutants with the same nominal Q_f value (-8) in the SF but generated by different combinations of aspartate and glutamate exhibit different electrophysiological properties. These results highlight the importance of both the architecture of the pore region and the electrostatic forces associated with the SF and extend our understanding of ion channel selectivity with significance for eukaryotic Na^+ and Ca^{2+} selective channels.

The work was supported by EPSRC (grant No.\ EP/M015831/1).

Posters

– 17. Membrane permeation: channels –

P-579

New insights in the translocation mechanism of ternary complexes of fluoroquinolones in *E. coli*M. Ferreira¹, C. F. Sousa¹, S. C. Lopes¹, L. Benier², M. Winterhalter², P. Gameiro¹¹REQUIMTE, LAQV, Departamento de Química e Bioquímica da Faculdade de Ciências da Universidade do Porto, Portugal; ²Department of Life Sciences and Chemistry, Jacobs University, Bremen, Germany

Fluoroquinolones (FQs) are antibiotics with a large spectrum of action against Gram (-) and some Gram (+) bacteria, known to penetrate the bacterial outer membrane across porins or through lipid/protein interface. OmpF is the main porin involved in the translocation of FQs in Gram(-) bacteria. Nevertheless, the misuse and overuse of antibiotics have triggered the development of bacterial resistance mechanisms against these drugs, specially through the decrease of the membrane permeability. The complexation of FQs with divalent metal ions and phenanthroline (phen) is a strategy to try to bypass this problem, as it forms stable complexes (metalloantibiotics) with different activity and a possible alternative influx pathway. Thus, we proceeded to study the interaction of several FQs and ternary complexes of copper(II)/FQ/phen with OmpF *Escherichia coli* total extract proteoliposomes, using fluorescence spectroscopy and surface plasmon resonance (SPR), under physiological conditions (T=37°C; pH 7.4). This study aims to evaluate the differences in the translocation of the FQs and the metalloantibiotics to try to understand if they could be a good choice to circumvent, at least, one of the resistance bacterial mechanisms used against FQs.

P-581

Membrane conductance of *Rhodobacter sphaeroides* and the input of F₀F₁-ATPase in its formationL. Hakobyan¹, L. Gabrielyan^{1,2}, A. Trchounian^{1,2}¹Research Institute of Biology, Yerevan State University, Yerevan, Armenia; ²Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia

The constant cycle of pump and leak of protons across the membrane contributes significantly to metabolic rate, particularly H₂ production by phototrophic bacteria. Current work is an attempt to reveal the input of F₀F₁-ATPase in membrane conductivity of *R. sphaeroides*, isolated from Armenian mineral springs. Proton conductance of bacterial membrane was measured at pH= 7.2, in the room temperature. Pulse doses of HCl rapidly decreased the extracellular pH, over time the decrease was partially compensated by proton flux across the membrane, alkalization of medium was detected. Cells treated with F₀F₁-ATPase inhibitor DCCD behaved similarly. Based on an estimated cellular buffering capacity the proton conductance of *R. sphaeroides* cells was 15 nmol of H⁺ /s/pH unit/mg protein. Addition of DCCD lowered this value, suggesting the involvement of F₀F₁-ATPase. However, under the conditions described, the decrease of proton conductance in the presence of DCCD was less than 10%, which shows the involvement of other proton leakage pathways. The role of membrane conductance in coupling mechanisms and bioenergetics of *R. sphaeroides* suggests it as a tool to interfere the H₂ production by these bacteria.

P-580

Non-equilibrium conduction through an open narrow ion channelW. A. T. Gibby¹, D. G. Luchinsky^{1,2}, A. Ward¹, I. K. Kaufman¹, P. V. E. McClintock¹¹Dept. of Physics, Lancaster University, UK; ²SGT Inc., USA

We present a new theory of conduction in biological ion channels, able to account for the extraordinary selectivity of the potassium channel which conducts K⁺ at the diffusion-limited rate (like an open hole) while obstructing other monovalent ions by up to 1000× [1]. It extends our equilibrium theory [2] to encompass non-equilibrium conditions, using master equations for steady-state occupancy probabilities of states in the channel, and takes account of electrostatic interactions, the difference between bulk and channel excess chemical potentials, the voltage drop between the bulk and the channel, and the bulk concentrations. It reproduces the Langmuir adsorption isotherm and Michaelis-Menten current saturation. Model predictions agree well with experimental data. The theory is also applicable to other narrow channels and to artificial nanopores.

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P-582

Real-time visualization of membrane nanopore formation by MACPF/CDC proteinsA. W. Hodel^{1,2}, C. Leung², B. J. Amelia³, N. Lukyanova², N. V. Dudkina², I. Voskoboinik³, H. R. Saibil², B. W. Hoogenboom^{1,2}¹London Centre for Nanotechnology, UK; ²Institute of Structural and Molecular Biology, UCL & Birkbeck College, London, UK; ³Peter MacCallum Cancer Centre, Melbourne, Australia

Proteins of the MACPF/CDC (membrane attack complex perforin/cholesterol dependent cytotoxicity) superfamily are effectors in pathogen attack and vertebrate immune defence. Released as soluble monomers, they can bind and self-assemble on a target cell membrane to form large transmembrane pores. Such pores can directly lyse and kill target cells, or allow transport of additional toxins. To study the assembly pathways of these proteins, we acquired high-resolution snapshots of by electron microscopy, and resolved membrane pore formation in real time by in-liquid atomic force microscopy. In our most recent work, we have elucidated mechanisms of pore formation by the MACPF protein perforin, a key mediator of lymphocyte cytotoxicity, used by our immune system to kill virus infected and cancerous cells. Our experiments reveal that the pore assembly proceeds via a short, membrane-bound prepore intermediate. These short oligomers can insert into the membrane and subsequently recruit additional prepore oligomers to grow the pore size. These results highlight the diversity of assembly pathways in membrane pore formation and provide molecular-scale insight into the mechanism of immune killing.

Posters

– 17. Membrane permeation: channels –

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***In vitro* characterization of functionally reconstituted ChIEF and ChR2**H. Hoi^{1,2}, Z. Qi^{1,2}, M. Gupta^{1,3}, C. D. Montemagno^{1,2}¹Ingenuity Lab, Canada; ²Department of Chemical and Materials Engineering, University of Alberta, Canada; ³Department of Electrical and Computer Engineering, University of Alberta, Canada; ⁴National Institute of Nanotechnology, Canada

Channelrhodopsins (ChRs) are light-gated cation channels whose activity can be optically controlled and are important tools in optogenetics. Understanding their functionality is crucial to decode the mechanism of the sensory photoreceptors, to guide their use in optogenetics, and to create new optogenetic tools. To study ChRs in a well-defined *in vitro* environment, we have overexpressed Channelrhodopsin 2 (ChR2) and ChIEF, an enhanced variant of ChR2, in *Pichia pastoris*. The reconstituted protein displayed the expected chromophore environment and secondary structure. The light-activated cation conductance of both ChIEF and ChR2 in artificial lipid bilayer was verified by fluorescent flux assay and planar lipid bilayer measurement. The later setup allowed us to observe explicit ion conductance in the reconstituted ChRs. In addition to the light-activated conductance, we observed a voltage-sensitive conductance in the dark state, which suggests the formation of a leaky pore in the nominally folded and functional protein. The reported results on the functionally reconstituted ChRs provide new insight to the molecular mechanism of the protein functionality and new guidelines to their use in optogenetics studies.

P-585

Energetics of polypeptide partitioning from the translocon into the lipid bilayer

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The heterotrimeric bacterial translocon SecYEG facilitates the secretion of hydrophilic proteins across the cytoplasmic membrane and the insertion of hydrophobic polypeptides into the membrane. Mounting evidence suggests that the energetic costs for protein partitioning into the membrane from (i) the aqueous lumen of SecYEG and (ii) the aqueous bulk solution are different. Here we tested the hypothesis that the restricted residence time of the nascent chain in the channel is responsible. Therefore, we first reconstituted SecYEG into planar lipid bilayers and stalled translocation intermediates of different hydrophobicities in the channel. We prevented back-sliding of the transmembrane segments out of the translocon by sandwiching them between the ribosome on one side of the membrane and calmodulin on the other. In the second step we forced SecYEG closure by applying a membrane potential¹. Hydrophobic transmembrane helices easily exited the lateral gate as indicated by zero residual ion conductivity, whereas hydrophilic ones remained in the region of the lateral gate or in SecYEG's lumen giving rise to ion conductivity. This approach allows assaying the cost for membrane partitioning at thermodynamic equilibrium. 1.Knyazev DG, et al. JBC 2014;289:24611-24616

P-584

Identification, alteration and recovery of chloride currents in the spinal-bulbar muscular atrophyA. M. Jiménez-Garduño¹, D. Michelatti¹, L. Tosatto², M. J. Polanco Mora², M. Basso², D. Arosio¹, M. Pennuto², C. Musio¹¹CNR, Institute of Biophysics, Trento, Italy; ²CIBIO, University of Trento, Italy

Spinal and bulbar muscular atrophy (SBMA) is a motoneuron disease caused by the expansion of a polymorphic CAG repeat encoding a polyglutamine (polyQ) tract in the androgen receptor (AR) gene, which leads to progressive muscle weakness and atrophy. PolyQ-AR is converted to a toxic species by binding to its natural ligands, testosterone or dihydrotestosterone (DHT). Our previous patch-clamp studies on motoneuron-derived MN-1 cells expressing SBMA polyQ repeats (MN100Q) have shown alterations in voltage gated ion channels (e.g. a clear reduction of inward and outward currents at increasing depolarizing potentials) that could be involved in the development of the disease. Following our dissection of ionic currents in MN-1 cells, we identified and characterized ClC-2-like chloride currents, whose amplitude were significantly reduced in the pathological form. Treatment with known rescuers of the disease, namely IGF-1 and PACAP, showed a recovery of the current amplitude indicating its possible role in the physiopathology of the disease. Thus we propose that ClC-2-like currents may be involved in the onset of the motoneuronal degeneration of SBMA; the recovering of chloride currents could be a possible target for new drug treatments of the disease.

P-586

Energetics of ion conduction in the NavMs channelE. Körpe¹, A. O. Acar¹, M. Çavuş², S. Kuyucak³, T. Baştuğ¹¹TOBB University of Economics and Technology, Turkey; ²Bozok University, Turkey; ³Sydney University, Australia

Voltage-gated sodium channels (Nav) are important targets for treating various diseases. Crystal structure of the bacterial voltage-gated sodium channel NavMs in the open conformation has been obtained recently. We used this structure in our simulation work in order to study channel stability, sodium ion coordination as well as the ion permeation mechanism. We have employed free energy techniques to calculate the potential of mean force (PMF) for ion movement through the NavMs channel. The PMF calculations revealed the ion-binding sites in the channel and the mechanism of ion conductance. We also studied tetrodotoxin binding to the NavMs channel. Using docking and molecular dynamic simulations, we have constructed a model for the NavMs-tetrodotoxin complex. The toxin binds to various parts of the channel and occludes the ion-conducting pore. Our results help to explain experimental data and provide insights into the Nav inhibition process. The complex structures we have found provide templates for developing new sodium channel blockers with improved affinity and selectivity properties, which will be useful in the design of novel drugs targeting sodium ion channels.

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P-587

Single-molecule investigation of directional transport through a bacterial transmembrane poreS. Lee¹, S. A. Ionescu¹, N. G. Housden², C. Kleanthous², H. Bayley¹¹Department of Chemistry, University of Oxford, UK;²Department of Biochemistry, University of Oxford, UK

Colicin E9 (ColE9) crosses the outer membrane of *E. coli* by passing through the trimeric OmpF porin. Entry is initiated by an intrinsically unstructured N-terminal domain (IUTD), which has two OmpF-binding sites (OBS). The IUTD threads through a first subunit of OmpF, reverses direction and enters a second subunit, leaving the IUTD sequence OBS2 within the first subunit, OBS1 within the second subunit and the intervening TolB-binding epitope in the periplasmic space [Housden et al., Science(2013)]. By using single-channel current recording, we examined the kinetics of IUTD entry. To monitor the directional transport of ColE9, we first established the orientation of OmpF in planar lipid bilayers using targeted covalent modification of introduced peripheral cysteine residues [Ionescu et al., ChemBioChem(2017)]. With a defined OmpF orientation, we studied IUTD translocation from the extracellular side of OmpF, which resulted in the occlusion of two of the three porin subunits. By measuring the binding rate constants for peptides representing the entire or parts of the IUTD domain, a kinetic model was derived for the initial translocation steps across the outer membrane. The final hooked conformation of the IUTD allows ColE9 to harness the pmf for transport across the inner membrane.

P-589

ERK modulates TRPV3 channel activity through the direct phosphorylation of threonine 264L. Macikova^{1,2}, L. Vyklicka¹, S. Boukalova¹, S. Chvojka¹, V. Vlachova¹¹Department of Cellular Neurophysiology, Institute of Physiology Czech Academy of Sciences, Prague, Czech Republic;²Faculty of Science, Charles University, Czech Republic

Transient receptor potential vanilloid 3 (TRPV3) is Ca²⁺-permeable nonselective cation channel widely expressed in keratinocytes where plays a critical roles in epidermal proliferation, differentiation, hair growth and a development of itch sensation. Genetic deletion of *trpv3* gene in mice leads to impaired epidermal barrier structure and strong deficits in responses to innocuous and noxious heat [1]. Furthermore, several gain-of-function mutations of TRPV3 result in severe channelopathy (Olmsted syndrome) accompanied by a strong skin defect. TRPV3 is physically associated into a complex with an epidermal growth factor (EGF) receptor whose activation via signaling pathway enables sensitization of TRPV3's responses. Here, we characterize the mechanism that leads to TRPV3 sensitization at the molecular level. Based on mutagenesis and whole-cell patch-clamp experiments on TRPV3 channels endogenously expressed in immortalized human keratinocyte cell line (HaCaT) and on transiently transfected HEK293T cells, we hypothesize that sensitizing effect of EGF on TRPV3 is mediated by stimulation of mitogen-activated protein kinase (MAPK, ERK) signaling pathway via ERK phosphorylation of threonine 264 in the N-terminal ankyrin repeat domain.

[1] X. Cheng et al.: Cell 141 (2010), 331-43.

P-588

Structural basis for NLP – plant membrane interactionT. Lenarčič¹, V. Hodnik^{1,2}, K. Pirc¹, M. Podobnik¹, I. Albert³, H. Böhm³, T. Nürnberger³, G. Anderluh¹¹Laboratory for Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Slovenia; ²Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; ³Center for Plant Molecular Biology – Plant Biochemistry, University of Tübingen, Germany

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are virulence factors that are secreted by several plant pathogens. They trigger leaf necrosis and immunity-associated responses in various dicotyledonous plants only. The molecular details of their cytolytic mechanism and peculiar specificity for dicot plants are unknown. We have identified glycosyl inositol phospho ceramide (GIPC), a plant-related sphingolipid, as a target molecule for NLP binding to plant membranes. Furthermore, we solved crystal structures of toxic NLP_{Pya} protein from pathogenic oomycete *Pythium aphanidermatum* in complex with glucosamine and mannosamine, respectively. Glucosamine often occurs at the site of glycosyl in the GIPC structure. Surface plasmon resonance showed that both sugars are able to bind to NLP_{Pya} by millimolar affinity. Structural analysis allowed us to determine the residues that participate in binding the sugar head-group and to elucidate the importance of the metal ion coordination due to its linkage to carbohydrate moiety through a network of hydrogen bonds. Collectively, this study provides important molecular insights into the novel mechanism of cytolytic activity of toxic proteins towards plant membranes.

P-590

Asymmetric rotational brownian motion on TRPV1 cation channel with X-ray single molecule techniqueK. Mio¹, K. Ikezaki², H. Sekiguchi³, T. Baba², Y. Matsushita², T. Kubo¹, Y. C. Sasaki²¹Operando-OIL and molprof, AIST, Japan; ²Graduate School of Frontier Sciences, The Univ. Tokyo, Japan; ³Japan Synchrotron Radiation Research Institute, Spring-8, Japan

The TRPV1 is a nonselective cation channel that responds to various signals including high temperature, low pH, as well as chemical compounds such as capsaicin and allylisothiocyanate. Recent progress in the cryoelectron microscopy and the single particle reconstruction technique enabled depiction of TRPV1 structure at near atomic resolution. However gating mechanisms of TRPV1 is not clearly understood because information about state-to-state transition is still missing. To understand the dynamics of TRPV1, we adopted the Diffracted X-ray Tracking (DXT) technique, in which individual protein was labeled with gold nanocrystals and the motion of X-ray diffraction spots from the gold crystal were investigated as intramolecular movement of TRPV1 in real time. We introduced “Met tag” for labeling nanocrystal and “His tag” for substrate absorption. Purified protein was immobilized on the Ni-NTA coated polyimide substrates. Data was three dimensionally (tilting, rotation, and time) analyzed. Brownian motion of outer helices was enhanced by capsaicin in dose dependent manner, detected as anisotropic rotation movement around the particle reflecting channel gating process. This movement was further compared to the high temperature evoked TRPV1 activation.

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Streptomycin entry into *Corynebacterium glutamicum* is mediated by the mechanosensitive channel MscCGY. Nakayama¹, K. Komazawa², N. Bavi^{1,3}, K. Nobata², K. Hashimoto², H. Kawasaki², B. Martinac^{1,3}¹Victor Chang Cardiac Research Institute, Australia; ²Tokyo Denki University, Japan; ³University of New South Wales, Australia

Streptomycin is a widely used antibiotic for the treatment of a number of bacterial infections, including tuberculosis. Bacterial cells exposed to streptomycin were shown to leak potassium and glutamate; however it is not clear how streptomycin causes the efflux of these osmolytes although it has been proposed that mechanosensitive (MS) channels may serve as potential pathways for streptomycin entry into cells. To address this question we have developed a novel giant spheroplast preparation of *Corynebacterium glutamicum* as a model of mycobacterial cells to investigate the behaviour of MS channels in the native membrane. Using patch-clamp, we identified two types of MS channels; MscCG, an MscS-like glutamate exporter, as well as an MscL-like channel, and examined their activation thresholds and gating kinetics. In addition, we determined mechanical properties of the *C. glutamicum* membrane by micropipette aspiration technique and found that its membrane is a very soft membrane. Moreover, we could demonstrate that the sensitivity to streptomycin depended on expression of the MscCG channels, but not MscL-like channels. Our study thus suggests that streptomycin interacts with and enters cells through MscCG channels in *C. glutamicum*.

P-593

New insights into Kir2.6 channel and its relation to thyrotoxic periodic paralysisR. M. Paninka, D. R. Mazzotti, E. Carlos-Lima, S. C. Lindsey, I. S. Kunii, M. R. Dias-Da-Silva, M. Arcisio-Miranda
Federal University of São Paulo - Unifesp, Brazil

Potassium Kir channels owe their name to its ability to allow inward K⁺ currents whereas the outward currents are blocked. Kir channels govern the resting potential of most cells and discipline the muscle excitability. Mutations in Kir channels cause disorders affecting the skeletal muscle. Recently, thyrotoxic periodic paralysis (TPP) has been related to Kir2.6 channel (*KCNJ18* gene). TPP is a neuromuscular disorder characterized by a three-way relationship among muscle weakness, hypokalemia, and thyrotoxicosis, the latter being essential for the crisis. Searching for Kir2.6 mutations in our TPP cohort, we distinguished two Kir2.6 isoforms, which haplotypes were named RRAI and QHEV, based on changes at amino acid residues. We also found two novel Kir2.6 mutations. *KCNJ18* cDNAs were cloned into mammalian expression plasmids and transiently transfected into HEK 293T cells to investigate the functional effects of Kir2.6 alterations. Patch-clamp and confocal laser scanning microscopy experiments were carried out. The haplotypes are equally functional and showed typical Kir currents. One mutation down-regulates the Kir2.6 activity, decreasing the K⁺ current density (~34%). This effect can be correlated with a substantial reduction of ~51% in membrane abundance relative to WT channel.

P-592

Studying lipid-mediated regulation of ion channels with dipole modifiers: a 10-year experienceO. S. Ostroumova¹, S. S. Efimova¹, A. A. Zakhharova¹, V. V. Malev^{1,2}, L. V. Schagina¹¹Institute of Cytology of the Russian Academy of Sciences, Russian Federation; ²Saint-Petersburg State University, Russian Federation

Here we reviewed our experience of application of low-molecular weight amphiphiles of dipolar nature, co-called membrane dipole modifiers, to study the influence of lipid environment on ion channels induced by various antimicrobials in lipid bilayers. Different flavonoids, phytoalexins, styryl and xantene dyes, local anesthetics, thyroid hormones, and PDE5 inhibitors were tested as dipole modifiers. At the first stage a detailed analysis of the relationships between the structure of modifying agents and changes in the dipole potential of membrane, its permeability to calcein, lipid domain structure and thermotropic behavior of lipids was carried out. Hereafter we found: 1) a key role of membrane dipole potential in the modulation of channels formed by antimicrobial lipopeptides and peptides: syringomycin E, surfactin, and cecropins; 2) dipolar nature of the gate mechanism providing cooperative opening of syringomycin channels; 3) heterogeneity of population of syringomycin channels which is determined by a mismatch of dipole potential of lipid ordered and disordered domains; 4) dipole modifiers could function as molecular chaperones that ensure the conformational stability of open or close state of lipopeptide and polyene channels. The study was supported by RFS (# 14-14-00565).

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Probing conformational changes of K⁺ channel KcsA by time-resolved homo-FRET studiesM. L. Renart^{1,2}, M. Giudici², J. A. Poveda², A. Fedorov¹, M. N. Berberan-Santos¹, M. Prieto¹, J. M. González-Ros², A. Coutinho^{1,3}¹CQFM and IN, I.S.T., Univ. de Lisboa, Lisbon, Portugal;²I.B.M.C, Univ. Miguel Hernández, Elche (Alicante), Spain;³Dep. Qui e Bioqu, Fac.Ciências, Univ. de Lisboa, Portugal

The K⁺ channel KcsA has been used as a model system to answer some of the long-standing questions about ion channels, including C-type inactivation. In this work, the highly conserved W67 residue was used as a reporter of the conformational dynamics of a KcsA mutant channel by performing polarized time-resolved fluorescence measurements. An analytical framework was first derived for analyzing homo-FRET among a homo-tetramer in a square geometry. A quantitative picture of the relative changes in inter-subunit distances at the level of the pore helices of the detergent-solubilized mutant KcsA under conditions favoring its closed/conductive and open/inactivated conformations was obtained by changing the ionic composition of the media or by site-directed mutagenesis approaches. The homo-FRET efficiency among the W67 residues was very sensitive to the ion occupancy of the mutant channel in the closed state. However, channel gating-linked inactivation induced by pH 4 produced only modest conformational changes of W67 KcsA at the level of its selectivity filter. These results will be discussed within the context of the molecular determinants of the fast inactivation process of the prokaryotic K⁺ channel. Acknowledgements: FAPESP/20107/2014.

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– 17. Membrane permeation: channels –

P-595

Role of aquaporins in hydrogen peroxide permeation and oxidative stressC. Rodrigues¹, F. Antunes², A. Čipak Gašparović³, G. Soveral¹¹Faculdade de Farmacia, Universidade de Lisboa, Portugal;²Faculdade de Ciências, Universidade de Lisboa, Portugal;³Institut Ruder Bošković, Zagreb, Croatia

Aquaporins facilitate membrane water and glycerol permeation and some AQPs also transport H₂O₂. Increased H₂O₂ permeation misbalance cell redox reactions and may induce tumorigenesis. To evaluate AQPs selectivity, we measured H₂O₂ permeability of yeast strains individually expressing mammalian AQPs with specific electrodes. The rAQP5-yeast strain showed the highest ability to permeate H₂O₂, that was confirmed by higher ROS intracellular accumulation after challenge with H₂O₂. Yeasts with increased levels of polyunsaturated fatty acids (PUFAs) showed induced AQP expression. AQP expression and production of PUFAs was investigated in transformed strains with $\Delta 12$ desaturase, where higher water permeability with low activation energy for water transport corroborated functional AQP expression. Additionally, the lipid peroxidation product 4-hydroxynonenal (HNE) showed to inhibit water permeability. AQP involvement in oxidative stress was confirmed in yeast overexpressing AQY1 and rAQP5. Sensitivity assays showed that both AQY1- and rAQP5-yeast strains were highly sensitive to H₂O₂ compared to controls, in accordance to the measured H₂O₂ transmembrane diffusion rate, thus suggesting that AQPs can be important players in oxidative stress resistance.

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Exploring the permeation of free and copper-complexed fluoroquinolones across the bacterial membraneC. F. Sousa¹, J. T. S. Coimbra², M. Ferreira¹, M. J. Ramos², P. A. Fernandes², P. Gameiro¹¹Requimte, LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Portugal;²Requimte, UCIBIO, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Portugal

Bacterial resistance is a critical public health issue and the development of alternatives to counteract this problem is urgent. Fluoroquinolones (FQs) are widely used antibiotics and bacterial resistance to them has been frequently reported. One important mechanism of resistance is the decrease of membrane permeability to FQs, by porin mutation. Previous studies suggest that the mechanism of permeation of FQ' copper complexes is different from the one of free-FQs, being porin independent and promoted by a strong drug-lipid interaction. In the present work, the mechanism of penetration in the bacterial cell of two FQs and their copper complexes is studied and compared by a combination of theoretical and experimental techniques. Molecular docking is used to explore the binding affinity between the drugs and the OmpF porin and molecular dynamics simulations are employed to analyse the free energies of both permeation pathways. A correlation between theoretical and experimental results is done. The findings of this work provide insights that will be helpful to proceed with the study of metal-complexes as alternatives to free-FQs in resistant infections.

P-596

Conformational changes of mVDAC1 upon tBid binding studied by pulse EPRK. Rudi¹, B. Mertins², L.-O. Essen², J. P. Klare¹¹Physics Department, University of Osnabrueck, Germany;²Departments of Chemistry and Biology, Philipps-University Marburg, Germany

Voltage-dependent anion-selective channels (VDACs) are the major proteins of the outer mitochondrial membrane of eukaryotes. They form membrane-spanning β -barrels and act as general diffusion pores for small hydrophilic molecules, adopting an open conformation at low or zero membrane potential and a closed conformation at potentials above 30–40 mV. VDACs are regulated by the interactions with other proteins and small molecules, and it is known that VDACs are conserved mitochondrial elements of the apoptosis pathway in both plant and animal cells.

We applied site directed spin labeling and distance measurements by double electron-electron resonance (DEER) spectroscopy to study the influence of tBid (truncated Bid), an activated BH3-only pro-apoptotic member of the Bcl-2 protein family, on mVDAC1. The results from pulse EPR spectroscopy are compared to simulated distance distributions obtained from molecular dynamics simulations of mVDAC1 in a lipid bilayer as well as in detergent.

We observed a clear influence of tBid binding on the inter spin distance distributions obtained for spin labeled mVDAC1, suggesting that interaction with tBid “closes” the anion channel by fixing the N-terminal α -helix within the pore.

P-598

Membrane permeabilization of egg phosphatidylcholine liposomes induced by cryoprotective agentsB. Sydykov¹, H. Oldenhof², L. Barros², H. Sieme², W. F. Wolkers¹¹Institute of Multiphase Processes, Leibniz Universität Hannover, Germany; ²Unit for Reproductive Medicine, University of Veterinary Medicine Hannover, Germany

Membranes are the primary site of freezing injury during cryopreservation or vitrification of cells. Addition of cryoprotectants can reduce freezing damage, but they can also disturb membrane integrity. In this study membrane destabilization induced by cryoprotectants (dimethyl sulfoxide (DMSO), dimethyl formamide, ethylene glycol, 1,2-propanediol and glycerol) was studied using carboxyfluorescein (CF) encapsulated into phosphatidylcholine liposomes as a model system. CF leakage was studied upon addition of the cryoprotectants as well as after freezing and thawing. Infrared spectroscopy was used to study interactions between the cryoprotectants and the lipid membranes. It is shown that all the protectants inhibit CF leakage during freezing, but only in a defined concentration range depending on the type of protectant. At higher concentrations all protective agents caused substantial leakage of the fluorescent dye already before freezing. Glycerol in particular caused CF leakage at relatively low concentrations, whereas DMSO was shown to be the least membrane destabilizing protectant. It is shown that DMSO dehydrates phospholipid head groups and raises the membrane phase transition temperature, whereas the other protectants do not.

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The sensor domain of TRPA1 channel regulates gating through a putative phosphoinositide-binding siteV. Synytsya¹, L. Zimova¹, A. Kadkova¹, L. Vyklicka¹, V. Zima², I. Barvik², V. Vlachova¹¹Department of Cellular Neurophysiology, Institute of Physiology Czech Academy of Sciences, Prague, Czech Republic; ²Division of Biomolecular Physics, Institute of Physics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic

Transient receptor potential ankyrin 1 (TRPA1) is a six-transmembrane domain (S1–S6) channel activated by a host of pungent and irritant chemicals that produce pain in humans. Its activation is thought to involve an allosteric mechanism whereby electrophilic agonists evoke interactions through the cytosolic domains and open the channel pore via an integrated nexus formed by intracellular membrane proximal regions that are densely packed beneath the lower segment of the S1–S4 sensor domain [1]. This part of the channel contains polar residues that form a uniquely charged crevice. Here we use molecular dynamics simulations combined with electrophysiology and systematic mutagenesis and identify conserved polar residues facing the putative lower cavity of the sensor domain as crucial determinants of the electrophilic, voltage and calcium sensitivity of the TRPA1 channel. We show that these amino acids may comprise a domain capable of binding membrane phosphoinositides such as phosphatidylinositol-4,5-bisphosphate, through which the channel's gating is regulated in a state-dependent manner.

[1] Paulsen CE, *et al.*, Nature, 520 (2015) 511–517.

P-601

Role of *Escherichia coli* formate channels in H₂ production during mixed carbon sources fermentationK. Trchounian^{1,2}, A. Trchounian^{1,2}¹Scientific-Research Institute of Biology, Faculty of Biology, Yerevan State University, Armenia; ²Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, Armenia

Formate is exported from the cytoplasm where, and in the absence of terminal electron acceptors, it is re-imported to the cytoplasm and converted to H₂. *Escherichia coli* has two formate channels encoded by *focA* and *focB* genes. FocA has an important role in regulating intracellular formate level during anaerobic fermentation. Role of FocB is not clear.

H₂ evolving activity in *E. coli* *focB* mutant grown on glucose and glycerol, in glucose supplemented assays, at pH 6.5 was determined to be ~ 2.5 fold lower than in wild type. In glycerol assays, H₂ producing activity in *focA* or *focB* mutants increased ~ 2.9 fold compared to wild type. To understand the role of formate in wild type H₂ producing activity, in the assays external formate at concentration of 10 mM was added. In formate assays, H₂ evolving activity of *focA* mutant was increased ~ 2 fold, but in *focB* it was decreased ~ 4.1 fold compared to wild type.

Taken together, these results have shown formate importing FocB activity by *E. coli* during mixed carbon sources fermentation. Exporting activity of FocA was observed in formate and glycerol assays. It means that both formate channels are active and play important role in regulating cytoplasmatic formate level.

P-600

Shaker-IR K⁺ channels gating in heavy water: role of structural water molecules in inactivation

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Molecular dynamic simulations revealed that the selectivity filter is sterically locked in the inactive conformation by buried water molecules in Kv channels upon inactivation. We found that extracellular heavy water (D₂O) dramatically slowed the entry into the inactivated state suggesting the role of these „structural water” molecules in the conformational stability of the selectivity filter. Alternatively, the effect of D₂O can be explained by the increased viscosity or an altered exit rate of K⁺ from the selectivity filter in a D₂O-based extracellular solution. Increased viscosity was mimicked by adding glycerol to the extracellular solution and determined the inactivation kinetics in various Shaker-IR channels that were transiently expressed in tsA-201 cells. Ionic current experiments were recorded from excised patches. The application of glycerol had negligible effect on the rate of inactivation kinetics. The exit rate of K⁺ ions was studied by changing the K⁺ gradient to allow the inactivation time constants to be determined for both outward and inward currents. Our results showed that extracellular D₂O did not change the $\tau_{\text{outward}}/\tau_{\text{inward}}$ ratio compared to control (H₂O on both sides). Our results support the idea that structural water molecules may have specific effects on inactivation kinetics by accessing to the region behind the selectivity filter.

P-602

Biophysical assessment of endothelial aquaporins as water and glycerol channels

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Aquaporins (AQPs, 0–12) facilitate water and glycerol (aquaglyceroporins – AQP3, 7, 9 and 10) permeation through membranes being crucial for water and energy homeostasis. In endothelia, the tissue that delivers blood components to all body, AQP1 is highly expressed and important for water permeation; however, less is known about aquaglyceroporins expression and function. In this study, human umbilical vein endothelial cells (HUVECs) were used to investigate aquaglyceroporins expression in endothelia. In addition, due to aquaglyceroporins involvement in several metabolic-related dysfunctions, their expression pattern and permeability were evaluated in a model of endothelial dysfunction.

Our data confirmed a high expression level of AQP1 and revealed, for the first time, that AQP3 is highly expressed in HUVEC; AQP7 and AQP10 were also detected, whereas AQP9 was absent. After induction of endothelial dysfunction, AQP3 mRNA and protein levels were not altered while AQP1 was diminished. Biophysical assessment of endothelial aquaporins showed that while glycerol permeability was unchanged in endothelial dysfunction, water permeability was 20% impaired, indicating that AQP1, but not AQP3, may have an important role in endothelial pathophysiology.

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In-silico opening of TRPA1 channel points to first extracellular linker as an open-state stabilizerL. Vyklicka¹, I. Barvik², V. Zima², L. Zimova¹, V. Vlachova¹¹Department of Cellular Neurophysiology, Institute of Physiology Czech Academy of Sciences, Prague, Czech Republic; ²Division of Biomolecular Physics, Institute of Physics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic

Transient receptor potential ankyrin 1 (TRPA1) is an excitatory ion channel involved in pain, inflammation and itching. This channel gates in response to many irritant and proalgesic agents, and can be modulated by calcium and depolarizing voltage. While the closed-state structure of TRPA1 has been recently resolved, also having its open state is essential for understanding how this channel works. Here we use molecular dynamics simulations combined with electrophysiological measurements and systematic mutagenesis to predict and explore the conformational changes coupled to the expansion of the presumptive channel's lower gate. We show that, upon opening, the upper part of the sensor module approaches the pore domain of an adjacent subunit and the conformational dynamics of the first extracellular flexible loop may govern the voltage-dependence of multimodal gating, thereby serving to stabilize the open state of the channel. These results are generally important in understanding the structure and function of TRPA1 and offer new insights into the gating mechanism of TRPA1 and related channels.

P-605

On application of Langevin dynamics to model Kv 1.2 channel gate activity – structure-based approachA. Wawrzekiewicz-Jalowiecka¹, L. D. Islas², Z. J. Grzywna¹¹Silesian University of Technology, Faculty of Chemistry, Department of Physical Chemistry and Technology of Polymers, Poland; ²Universidad Nacional Autonoma de Mexico, School of Medicine, Mexico

Kv 1.2 channels are voltage-dependent and highly K⁺-selective ion channels. They are involved in many important biological processes (e.g. excitation of sensory neurons). In spite of the existence of many reports considering structure-function relations of the Kv 1.2 channel's domains in literature, some aspects of their activity remain ambiguous.

We propose a structure-based, mesoscopic model of the Kv 1.2 channel gating, and determine to what extent voltage-dependent geometry of its inner vestibule may affect the observable probability of the channel's open state. We use open structures of this channel obtained in our previous geometric studies as a template, and carry out simulations of four fluctuating, pore-forming S6 domains. The dynamics of their motions is determined by the Langevin equation in a potential field describing both possible interactions between the S6 helices and S4-S5 linkers, and hydrophobic mutual interactions between inner parts of neighbouring S6 segments. We analyse also an impact of additional factors that may affect open-closed fluctuations of the channel like: spontaneous rotations of the side chains of S6 helix around the single bond linking them to the backbone, membrane fluctuations and internal strains within the S6 segment.

P-604

Mechano- and thermosensitivity of the BK potassium channelsA. Wawrzekiewicz-Jalowiecka¹, B. Dworakowska², Z. J. Grzywna¹¹Silesian University of Technology, Faculty of Chemistry, Department of Physical Chemistry and Technology of Polymers, Poland; ²Warsaw University of Life Sciences, Department of Physics, Division of Biophysics, Poland

Large-conductance, voltage dependent, Ca²⁺-activated potassium channels (BK) are transmembrane proteins that control such processes like: smooth muscle spasms, hormone secretion, excitation of neurons. Here, we investigate to what extent temperature (in the range of 17–37 in degrees Celsius with $\Delta T = 5$ Celsius degree step) and membrane strain (generated by suction on the patch clamp pipette of $p = 0 \div 40$ [mmHg] with a $\Delta p = 10$ [mmHg] step) are regulating parameters for channel gating at membrane depolarization and hyperpolarization.

Basing on the obtained patch clamp results, kinetic, thermodynamic and correlation analysis of channel gating was carried out. The obtained results indicate that considerable quantitative differences between dependencies of channel's characteristics (e.g. open state probability, open and closed dwell-times, conductance) on temperature and pressure are evident for different regimes of voltage. We present the inferences about possible effects of membrane fluctuations and morphology on channel's activity in the light of current research.

P-606

Simplifying artificial bilayer experiments: Single-molecule experiments on micro-cavity arraysC. Weichbrodt¹, J. Wang¹, M. Kreir¹, E. Zaitseva², G. Baaken², N. Fertig¹¹Nanion Technologies GmbH, Ganghoferstrasse 70a, 80339 Munich, Germany; ²Ionera Technologies GmbH, Herrmann-Herder-Strasse 7, 79104 Freiburg, Germany

Artificial lipid bilayers represent the gold standard for the investigation of membrane spanning species like purified ion channels or membrane-active species in general like toxins. However, the convenient and reproducible preparation of these model bilayers as well as the delicate successive handling for data acquisition still remain an obstacle for the trouble-free introduction of this technique.

We here present Nanion's Orbit systems that are explicitly designed to meet the special requirements of experiments on artificial bilayers. Use of Ionera's MECA (*micro electrode cavity array*) chip technology combined with state of the art low noise amplifiers (Elements S.R.L.) enables the fully parallel low-noise recording of four or even 16 separate lipid bilayers at bandwidths up to 100 kHz. The systems have already been validated with targets as diverse as ligand and voltage gated ion channels, porins and origami DNA constructs, antimicrobial peptides or membrane active toxins. The optional temperature control for the Orbit mini furthermore allows for experiments on temperature sensitive species such as TRP channels or for experiments at physiological temperatures whereas the fully automated bilayer generation on the Orbit e16 further improves the system's usability.

Posters

– 17. Membrane permeation: channels –

P-607**Ca²⁺ permeability of NaChBac heterotetramers can explain the EEEE paradox in bacterial Na⁺ channels**

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The highly selective permeation of ions through voltage gated Na⁺ and Ca²⁺ channels can be explained in terms of fixed negative charged in the pore region (Q_f) and has been described in terms of ionic Coulomb blockade (CB). However, the same electrostatic charge and structure in the channel pore region (i.e. “EEEE motif”) is reported to be responsible for both Na⁺ conduction in bacterial channels and Ca²⁺ conduction in mammalian channels. To address this anomaly, we considered the additional charged residue (D) conserved in domain II of L-type Ca²⁺ channels and speculated that Ca²⁺ selectivity in these channels is actually defined by an EEEED locus ($Q_f = -5$). To test this hypothesis, we recorded Na⁺ and Ca²⁺ currents from *heterotetramers* of NaChBac mutant channels in which the Q_f value ranged from -4 to -8. Attempts to concatenate NaChBac were unsuccessful but transfection of cells using defined mixtures of cDNA encoding for NaChBac mutants exhibiting different Q_f values revealed that a $Q_f \geq -5$ is necessary for Ca²⁺ permeation and are consistent with the hypothesis that Ca²⁺ permeation in L-type Ca²⁺ channels is mediated by a EEEED locus.

The work was supported by EPSRC (grant No.\ EP/M015831/1).

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Posters

– 18. Modelling, inference, big data –

P-608 (O-109)

Transforming protein sequence and composition into numbers: A BIG DATA analysis tool for proteomes

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Protein sequences represented as strings of alphabetical characters are poorly suited for BIG DATA analysis of the vast proteome information. Further, the physicochemical properties of amino acids are lost by such representation. Here we present a novel approach, addressing the twin issues above by coding each natural amino acid in protein sequence as a prime number. The magnitude of assigned number was based on the hierarchy of the amino acid in the hydrophobicity scale. For example, most polar arginine was 2, while least polar isoleucine was 1831. As prime factors of any integer are unique, the inherent advantages of this numerical transformation are manifold, namely: a) Amino acid composition of any sequence can be stored as prime product (ProtID) of all residues in sequence; b) Base 2 logarithm of ProtID yields PS-Score that can serve as an identity tag of protein sequence composition, besides providing ready information on average residue polarity in the sequence; c) Coarse grain representation of protein sequence in a contiguous block of 3/5/10 residues is possible as local ProtIDs; d) Finally all proteins in a given proteome can be sorted based on their PS-Score values. Insights gained from analysis of proteomes ranging from bacteria to humans, shall be presented.

P-610 (O-107)

Neuronal signaling pathways estimated from whole-brain imaging data of *C. elegans*Y. Iwasaki¹, T. Teramoto², S. Oe², T. Tokunaga³, O. Hirose⁴, S. Wu⁵, Y. Toyoshima⁶, M. S. Jang⁶, R. Yoshida⁵, Y. Iino⁶, T. Ishihara²

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The nematode *C. elegans* is a useful model organism in neurobiology because the synaptic connectivity between all the 302 neurons has been completely identified. Recently, we developed a high-speed 4D calcium imaging system to measure neuronal activities in the whole central nervous system of *C. elegans*. We estimate neuronal signaling pathways in the whole central nervous system from the imaging data. In *C. elegans*, the result is able to be compared with the identified synaptic pathway. Neuronal signaling pathway is determined by the correlation coefficient and the Granger causality test. The correlation coefficient is commonly used to quantify undirected influence between two data. On the other hand, the Granger causality test quantifies directed influence ("predictive causality"). We find that actually used signaling pathways are different from the synaptic pathways in *C. elegans*. Some causalities between two neurons are derived from detour routing via the third neuron but not via two or more neurons. Causality network consists of the synaptic pathways within the second nearest neighbor distance. The transfer entropy, which is another causality detection based on information theory, is also discussed.

P-609 (O-108)

Colonization dynamics of bacteria in miceF. Bansept¹, E. Slack², R. R. Regoes³, R. Voituriez¹, C. Loverdo¹

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Our work aims at developing a stochastic model for the colonization of an organism by bacteria, applied to experimental data on mice gut colonization by the salmonella. We work both analytically and by simulations to extract biologically relevant parameters – like growth and death rates – from the indirect experimental data, which consist of the final proportions of genetically tagged bacteria. For some experiments, the results obtained using different observables characterizing the tags distribution are not compatible. We tested a model with two subpopulations of bacteria growing at different rates and showed that it allows to get qualitatively closer to the experimental observables, even if it is still quantitatively insufficient.

In experiments on the effects of a vaccination on this system, while the total number of bacteria is similar, the tag distribution is more variable in the case of previous vaccination or when immunoglobulin A is given to the mice, than in the absence of such a treatment. When immunoglobulin A is present, daughter bacteria remain stuck together after division. This leads to a smaller effective bacterial population size. When implementing this in the model, we could show that it could indeed explain the experimental tag distribution.

P-611

MDbox: a cloud-based repository for molecular dynamics simulationsK. Condic-Jurkic¹, M. Gregson²

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Computational techniques such as molecular dynamics (MD) simulations are increasingly used to study the interactions of biomolecules and/or materials at an atomistic level. MD simulations are still computationally costly, requiring super-computer access and significant scientific input. We are currently developing MDbox – a prototype for an open access repository for MD simulation datasets. MDbox aims to provide a platform for storing and sharing trajectories and their corresponding input files, which should improve documentation of commonly used protocols and enhance the replicability and reproducibility of simulations. Ultimately, MDbox should make collaboration and data exchange easier and provide an alternative for making research publicly available and citable.

In our information-driven era, this open data approach is of tremendous value for further development of computational modelling and for cross-disciplinary researchers in both academia and industry. The ability to access a large number of simulations in a single repository will create unprecedented opportunities for research into "big data" analysis and mining techniques.

Posters

– 18. Modelling, inference, big data –

P-612

Understanding cancer phenomena using a thermodynamic-based approach

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We seek to address fundamental questions in cancer biology by an experimental-theoretical approach based on physico-chemical laws. We have recently pioneered the application of the thermodynamic-based surprisal analysis, which has been previously applied to systems in chemistry and physics, to biological processes. We have shown that through the accurate resolution of the protein networks that deviate the cancer system from its balanced state, various biological phenotypes can be predicted. For example, we have demonstrated that using a thermodynamic-based proteomic analysis in varying cell-cell distances, the direction of movement of brain cancer cells can be predicted and experimentally manipulated.

Here we present single cell and bulk proteomic methods integrated with thermodynamic-derived information theory. We demonstrate how complex biological phenomena, such as cellular tumor architectures or inter-tumor variability can be modeled using a limited number of key physical parameters. Furthermore we show how these parameters are used to predict cellular architectures or to design high-precision, patient-specific drug cocktails. Generally speaking, this approach provides a framework that models biological systems in order to learn how to predict and manipulate their behavior.

P-614

Protein Data Bank Japan (PDBj): updated semantic web services and tools for large structures

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The Protein Data Bank Japan (PDBj, <https://pdbj.org>), a member of the worldwide Protein Data Bank (wwPDB), accepts and processes the deposited data of experimentally determined macromolecular structures with the OneDep system (Young et al., Structure 25, 536-545, 2017) for depositors in Asia and Middle East regions. While maintaining the archive in collaboration with other wwPDB partners, PDBj also provides a wide range of services and tools for analyzing structures and functions of proteins. To enhance the interoperability of the PDB data with other life science databases, we have also developed the semantic web services with PDB/RDF, PDB data in the Resource Description Framework (RDF) format, which is now a wwPDB standard called wwPDB/ RDF. We have enhanced the connectivity of the wwPDB/RDF data by incorporating various external data resources. Services for searching, comparing and analyzing the ever-increasing large structures determined by hybrid methods are also described (Kinjo et al., Nucl Acids Res. 45, D282-D288, 2017).

P-613

Comparative and synergetic analysis of membranotropic effect of the PLA2s of MLO snake venom

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It is known that snake venoms are a complex of proteins and the interaction of different venom components with the membranes is not always the same. The aim of the proposed investigation is to obtain detailed information about the mechanism and topology of the separate component of the *Macrovipera lebetina obtusa* (MLO) venom in the membrane-binding process. We chose PLA₂ as the focus of the study. Equally important, we aim at investigating the impacts of such interaction changes on the properties of membrane (GUVs and erythrocytes ghost). At the same time we identified the synergetic action of these two monomeric PLA₂ in the membrane. We used 6-lauroyl-2-dimethylaminonaphthalene (LAURDAN) and 6-propionyl-2-dimethylaminonaphthalene (PRODAN) fluorescence probes which allowed us to determine the membrane polarity more accurately using a generalized polarization function (GP). Our results show that two types of PLA₂ bring viscosity reduction in GUVs membrane and it is more potent when these PLA₂ were together. In case of the erythrocytes ghost we have not observed any significant difference.

P-615

Physical Model of Collective Cell Migration in Zebrafish Gastrulation

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During zebrafish gastrulation, Dorsal Forerunner Cells (DFC) move as a collective group in close association to the overlying Enveloping Cell Layer (EVL) towards the vegetal pole. Two interesting features of this process are the collective migration and clusterization, which may be related to the physical link between DFCs and EVLs, which is gradually lost in the process. We developed a physical model to understand the role of DFC-DFC adhesive interactions and physical attachment to the EVL in the process. The model applies long range interaction potential to simulate attractive and repulsive interaction forces, random walk to simulate stochastic cell migration through protrusions, and elastic spring for EVL attachment. Initial condition, DFC division rate, detachment rate, EVL speed, effective diffusion coefficient are derived from experiments, while potential coefficients are free parameters.

The simulation results show that there is a set of parameters where there is good agreement with the experimental observations is obtained. Additionally, simulations show that all the elements of the model are necessary to properly describe the collective migration and clusterization.

Posters

– 18. Modelling, inference, big data –

P-616**Identifying the Interaction Site of Poly ADP-ribose Polymerase-4 with NAD by Using Molecular Dynam**A. Unlu¹, B. Dinc², M. Budak¹, T. Sipahi¹, M. Bektaş³, R. Nurten³¹Trakya University, Turkey; ²Istanbul Kemerburgaz University, Turkey; ³Istanbul University, Turkey

The PARPs are ubiquitous enzymes in most eukaryotes and are known to function in post translational protein modifications where the ADP-ribose moiety is transferred from NAD onto specific substrates. Seventeen putative PARP sequences have been identified in the human genome, including at least six enzymes: PARP-1, PARP-2, PARP-3, PARP-4, PARP-5a, and PARP-5b. Excessive activation of PARPs can cause the depletion of NAD⁺ and ATP in the cell, besides inflammatory injury, cell dysfunction, and ultimately necrotic cell death. In our study we defined the three-dimensional structure of PARP-4 whose molecular structure has not been determined yet by using the method of homology modeling. We made an interaction simulation of the structure obtained with NAD in computer using the docking method. Thus, we determined the hot spots of both structures. We used the scoring methods such as XP Glide and SP Glide which was developed by the institute of Max Planck to determine amino acids with the highest binding energies at the hot spots detected. During the studies of minimizing the energy required for the structures defined, we used the simulation programs of NAMD and CHARMM. We made long-term calculation processes through the TR-DATA Grid computing systems within the structure of TUBITAK.

P-617**Human and Neanderthal HORs in NBPF genes chromosome 1– DNA sequence difference and similarities**I. Vlahović¹, M. Glunčić¹, M. Rosandić², I. Martinjak^{1,2}, V. Paar^{1,2}¹Department of Physics, Faculty of Science, University of Zagreb, Bijenička c. 32, 10000 Zagreb, Croatia, Croatia; ²Croatian Academy of Sciences and Arts, 10000 Zagreb, Croatia, EU, Croatia

With availability of using Neanderthal sequenced DNA data, we have opportunity to identify differences on molecular level in comparison to human DNA. Using computational Global Repeat Map – GRM, we can identify various types of repeats that exist in DNA sequences. Our focus is on identification of higher order repeats – HORs (tandem within tandem repeats in tail to head fashion) and their comparison in different or same species. With this poster, we show differences in HORs in chromosome 1 in NBPF genes, genes responsible for human brain development and neurodegenerative diseases such as schizophrenia, autism, microcephaly, macrocephaly and neuroblastoma. We identify six HORs in Neanderthal and five HORs in human NBPF gene based on monomers length ~1600 bp (making 3mer HOR structure ~4770 bp), while in primates those kind of structures still have not been identified. Divergence among HORs in each array are in human less than in Neanderthal arrays ~1.68% vs. 6.16%. We conclude that those HOR structures are older in Neanderthal than in humans. Finding HORs structures in both species could reveal differences between them and eventually give an answer why we, as human species, did survive.

Posters

– 19. Experimental and computational approaches to protein design –

P-618 (O-113)

Designing artificial TIM-barrel proteins from scratch: the Octarellin model

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De novo protein design is a growing field in protein chemistry, where artificial proteins are first designed *in silico* and then validated experimentally. Our group has a long tradition in the design of artificial (β/α)₈-barrel proteins, known as Octarellins [1]. This fold, also known as TIM-barrel, is widespread in nature, particularly in enzymes, and represent an interesting target for therapeutic or biological applications. Here we present a novel protocol for the *de novo* design of TIM-barrels.

Both Rosetta and Modeller modeling softwares were used to create the backbone structure of a TIM-barrel fold and to generate more than 10.000 artificial amino acid sequences. Stability was tested for the more interesting models by running molecular dynamics (MD) simulations, using GRO-MACS. The best models were chosen for protein production and preliminary biophysical characterization.

The design of artificial proteins and the improvement of bioinformatics tools for protein modeling, structure prediction and MD simulations seem to be essential for a comprehensive knowledge of protein structure in general, as well as for an optimal use of the massive amount of data resulting from the numerous genome sequencing projects.

1. Figueroa, M. et al., (2016), *J. Struct. Biol.*, 195, 19–30.

P-620

The biophysical characteristic of four [4Fe4S] cluster types coordinated by protein maquettes

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Iron-sulfur clusters (FeS) play an important role in several metabolic pathways because of their redox properties. There is possibility to introduce such a cluster in *de novo* designed proteins, which may be used in biotechnology. Here we are studying if and how the redox properties of FeS cluster depends on its position in the protein. We used four types of *de novo* designed proteins: assembling FeS by cysteine residues form symmetric loops, with FeS bound in the hydrophobic core, with FeS in the single asymmetric loop and with assembled by four cysteine residues from terminal parts of homodimeric peptide. FeS cluster was reconstituted *in vitro* from simple chemicals and had typical UV/VIS spectrum (415 nm maximum and an arm at about 350 nm). We immobilized the holoproteins on carbon electrodes getting directly electrochemical characteristics, which were then compared with values obtained from spectrophotometrical titration. The FeS bound into hydrophobic core has higher midpoint potential, while the other where comparable.

Our research will allow to apply the optimal motifs for FeS binding in artificial electron pathways construction. The project granted on the decision number DEC-2013/09/B/NZ1/01111 by the National Science Centre, Poland.

P-619 (O-114)

Function conversion between CPD and (6-4) photolyases

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Photolyases (PHRs) are DNA repair enzymes with two different types: CPD PHR repairs cyclobutane pyrimidine dimers (CPDs), while (6-4) PHR repairs (6-4) photoproducts ((6-4) PPs). The features distinguishing the substrates of CPD and (6-4) PHRs are not well understood. In this study, we attempted functional conversion between CPD and (6-4) PHRs by monitoring distinct repair signals by FTIR spectroscopy. We found that a triple mutant of (6-4) PHR can repair the CPD. In contrast, the (6-4) PP was not repaired by the reverse triple mutation of CPD PHR, even after eight more mutations were added. The observed asymmetric functional conversion is interpreted in terms of more complex repair mechanism for (6-4) PP, which was supported by quantum chemical/molecular mechanical calculation. Furthermore, we scrutinized their amino acid sequences and narrowed down the amino acid positions important for distinguishing the substrates for PHRs. Specifically, we built an alignment and compared the highly conserved positions in each PHR. The differences in the conservation pattern of amino acid residues can be an explanatory cue for the difference in the function of PHRs.

P-621

Calixarene glue facilitates crystal structure determination of *Penicillium* antifungal protein

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Diffraction-quality crystals are essential for X-ray crystallography, and protein crystallization can be a serious bottleneck. ‘Molecular glues’ offer a strategy to overcome this bottleneck. Polar and/or charged compounds such as *p*-sulfonatocalix[4]arene (scx₄) can interact with surface residues via non-covalent interactions and promote crystallization. Here, scx₄ was used to crystallize *Penicillium* antifungal protein (PAF) for which no crystal structure has been reported to date. Crystallization of the PAF – scx₄ complex yielded crystals that diffracted to a resolution of 1.3 Å (PDB 5MWS). Scx₄ binds to Lys30 and neighbouring residues. An NMR characterization revealed significant chemical shift perturbations for these residues suggesting that the same scx₄ binding site occurs in solution. Crystallization of PAF was also attempted with scx₆ and scx₈, to study the influence of increasing calixarene size on protein-ligand interactions and protein assembly. Crystals of PAF – scx₆ and PAF – scx₈ diffracted to a resolution of 1.3 Å and 1.5 Å, respectively.

Posters

– 19. Experimental and computational approaches to protein design –

P-622

Introducing functionality into hyperstable coiled-coil scaffoldsA. L. Boyle¹, M. Xiao¹, N. Crone¹, G. Rhys², P. Voskamp¹, N. Pannu¹, A. Kros¹¹Leiden University, Netherlands; ²University of Bristol, UK

We have utilised a *de novo* designed coiled-coil scaffold containing only the minimum information required for structural specificity and redesigned it to incorporate metal-binding functionalities. This approach allowed us to: determine the modifications necessary to effect metal-sensitivity in such a scaffold; investigate whether the structure of the peptide was altered by including metal-binding residues; and to investigate metal-binding affinity and specificity. Several constructs were iteratively designed and subsequently tested for their ability to bind metals using a variety of biophysical techniques including circular dichroism (CD) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and analytical ultracentrifugation (AUC) in addition to ongoing crystallographic studies. We found that multiple modifications were necessary to effect metal binding and, interestingly, a high degree of selectivity for certain metal ions was observed when compared to other, similar, peptide scaffolds. The structural basis for this selectivity is currently under investigation and it is anticipated that such selectivity would make these structures attractive for applications such as biosensing and metal-induced oligomerization of biomolecules.

P-624

Designing a novel biocatalysis platform based on B-peptide linkagesR. M. Crean^{1,2}, M. van der Kamp⁴, J. Mason², S. Bull³, C. R. Pudney²

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High activity and selectivity under mild conditions, the green credentials of enzymes are clear. The same evolutionary pressure responsible for producing enzymes with these beneficial characteristics is also responsible for making them highly sensitive to their environment, and often in need of stabilisation before commercial application can be considered. The oligomerisation of a diverse array of building blocks can produce defined secondary structural units akin to that of α -amino acids. To this end we have used a combination of computational and rational design to stabilise two small all α -helical enzymes by the incorporation of β -cyclic amino acids. A range of biophysical techniques are currently being employed to understand the effect these mutations have caused. To complement, molecular dynamics simulations of the wild-type and variants structures are being run to simulate the changes in stability at the atomistic level.

P-623

Structural and computational analysis of human ketohexokinaseA. Chang, W. Rabeh, S. Kirmizialtin
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Human Ketohexokinase (KHK) catalyzes the phosphorylation of fructose to fructose-1-phosphate, using ATP as the phosphate donor. The processing of dietary fructose by KHK is unregulated and, in combination with a sedentary modern lifestyle, has led to an increased incidence of metabolic syndrome. The phosphorylation reaction is dependent on the presence of Mg^{2+} , however the positions of the Mg^{2+} ions have not been determined crystallographically. Using MD simulations and homology modeling we determined two Mg^{2+} ion binding sites, however our simulations suggest that only one Mg^{2+} ion is tolerated due to electrostatic repulsion that leads to rapid dissociation of the substrates. To benchmark our results we performed simulations in two previously known disease-causing mutations of KHK (G40R and A43T). The G40R mutation prevents fructose binding by causing the enzyme to undergo a wide-open state and, hence, rapid release of its substrates before chemistry can occur; the A43T mutation results in an active site rearrangement that blocks the fructose binding. Based on the one- Mg^{2+} model, we studied the free energy of substrate binding and their optimal order. While both substrates can bind the free enzyme, our data suggests that binding ATP- Mg^{2+} then fructose is the optimal mechanism.

P-625

In-vivo assembly and characterization of CCIS, an in-silico designed [4Fe-4S] cluster proteinB. P. Jagilinski¹, V. Nanda², D. Noy¹

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Iron-sulphur (Fe-S) clusters proteins are metalloproteins that function as electron carriers, and play an important role in many biological redox processes. Those containing four-iron four-sulphur [4Fe-4S] clusters are of interest to the development of alternative fuels such as molecular hydrogen. Our goal is to design artificial [4Fe-4S] clusters proteins that will serve as custom redox carriers by using computational tools. Developing an efficient system for assembling the [4Fe-4S] cofactors within the protein scaffold is critical to this endeavour. It is challenged by the inherent oxygen lability of [4Fe-4S] clusters. The Coiled-Coil Iron-Sulphur protein (CCIS) was computationally designed as a minimal, non-natural protein scaffold incorporating an active [4Fe-4S] cluster. Here, we report on the heterologous expression in *E. coli*, and *in-vivo* assembly of [4Fe-4S] clusters within the CCIS holo-protein. This was achieved by supplementing iron and sulphur additives to the medium during the induction phase. To ensure the stable assembly of [4Fe-4S] clusters, the entire CCIS purification is carried out strictly under anaerobic conditions inside a glove box. The assembled Iron-sulphur (Fe-S) clusters were characterised by chromatography, optical and magnetic spectroscopy techniques.

Posters

– 19. Experimental and computational approaches to protein design –

P-626

Rational design of SAKe-CuP proteins

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Recently we have developed the computationally designed Pizza proteins. These proteins have a 6 fold symmetric beta-propeller architecture. The protein is highly stable and a derivative version is able to biomineralize the smallest nanocrystal reported to date. The protein however lacks a clear binding site that prevent the coordination of different complexes. Therefore our aim was to create a novel symmetric protein that has putative functionisable loops. These features are present in the 6-fold pseudo-symmetric keap1 KELCH repeat proteins. Here we report the design of a novel Self-Assembling-Kelch based Cuplike Protein (SAKe-CuP). Multiple designs have been expressed and structurally validated.

P-627

Protein oligomerization triggered by anionic calixarene macrocyclesM. L. Rennie¹, A. M. Doolan¹, C. L. Raston², P. B. Crowley¹¹School of Chemistry, National University of Ireland Galway, Ireland; ²School of Chemical and Physical Sciences, Flinders University, Adelaide, Australia

Enzymes and other proteins exhibit hugely diverse molecular functionalities. Hijacking these properties into bionanomaterials provides tantalizing new prospects in nanotechnology. The first step generally requires engineering of proteins into assemblies¹; a useful avenue involves surface recognition via supramolecular building blocks. Lysine binding by water-soluble calixarenes has previously been highlighted as a route to solid state assembly of proteins². Here, we provide a proof of principle that anionic calixarenes can be used to control the solution state assembly of cationic proteins. Size-exclusion chromatography coupled to static light scattering was used to show that phosphonated calix[6]arene drives dimerization of cytochrome *c*, while sulfonated calix[8]arene drives tetramerization. X-ray crystallography and NMR were used identify the probable structures and assembly mechanisms. Importantly, these ligands are of comparable size to cytochrome *c* and bind with low micromolar affinity, the resulting complexes may therefore yield insights into protein-protein interaction surfaces.

1. N. P. King *et al*, *Nature*, **2014**, 510, 103.2. R. E. McGovern *et al*, *Nat. Chem.*, **2012**, 4, 527.

P-628

The multiple origins of the hydrophobicity of fluorinated apolar amino acidsJ. R. Robalo¹, S. Huhmann², B. Kokschi², A. Vila Verde¹¹Max Planck Institute for Colloids and Interfaces, Germany;²Freie Universität Berlin, Germany

Fluorinated amino acids – *e.g.* amino acids where the side chain methyl groups are substituted by trifluoromethyl groups – have been shown to increase the thermal stability of proteins. The proposed origin of this effect stands on the greater hydrophobicity of fluorinated amino acids *versus* their non-fluorinated counterparts, but how fluorination alters the hydrophobicity of molecules is, as of yet, also not entirely understood. In this work, we evaluate amino acid hydrophobicity *via* molecular dynamics simulations using a force field we developed. We observe that the impact of fluorination on hydrophobicity is not only site-dependent but also related with the configuration of the fluorination site. We quantify the contribution of each mechanism to the changes in hydration free energy incurred on by fluorination using a simple phenomenological model. We conclude that a decrease in the number of backbone-water hydrogen-bonds is the main responsible for the less negative hydration free energy of fluorinated amino acids. The hitherto non-existing force field and atomistic level, quantitative, explanation of the origins of the hydrophobicity of fluorinated amino acids provide a route to understand and predict the unique physical-chemical properties of fluorinated proteins.

P-629

Exploring Gd(III) and Cu(II) binding in coiled coil peptides with EPR distance measurementsA. Shah¹, L. N. Slope², A. S. Kooduthurai², J. E. Lovett¹, A. F. A. Peacock²¹School of Physics and Astronomy, University of St Andrews, St Andrews, KY16 9SS, UK; ²School of Chemistry, University of Birmingham, Birmingham, B15 2TT, UK

Electron paramagnetic resonance (EPR) is an incredibly versatile technique used to analyse systems containing paramagnetic centres, including intrinsic metal centres, metal labels and radical spin labels that are covalently bonded to cysteines. Hyperfine spectroscopy, one of the subsets of EPR, can be used to determine structural information about binding sites, while nanometer distance distributions can be determined by analysing the dipolar interaction between pairs of paramagnetic centres using a DEER experiment. EPR spectroscopy is capable of measuring at nanometer scale with a high degree of accuracy and this makes it an important biophysical tool for investigating biomolecules, and particularly protein structure.

Here we will demonstrate that the coiled coil peptides can be designed to hold two Gd ions by measuring distances between the intrinsic Gd pairs using DEER. We then show that the same distances can be measured if Cu ions are present rather than Gd. Hyperfine spectroscopy was used to probe whether the Cu are directly bound to nitrogen atoms and negative results further strengthen the case that the Cu is being bound in a wholly hard oxygen peptide environment. To the best of our knowledge, this is the first example of this coordination for Cu in proteins.

Posters**– 19. Experimental and computational approaches to protein design –****P-630****Computational design of symmetric protein building blocks**

A. R. D. Voet, H. Noguchi, J. P. M. Vrancken, S. Wouters, B. Mylemans
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The modular nature of the protein architectures suggests that proteins have evolved by the duplication and fusion of genetic fragments to give rise to modular, often symmetric protein architectures, which later diversified under the influence of evolutionary pressure. We have developed a computational protein design work-flow in which we REverse Engineer Evolution (RE3Volutionary) to create symmetrically self assembling functional protein building blocks.

Our computational protein design workflow has been successful for the design of different protein building blocks (Pizza with 6fold, Tako with 8 fold, Ika with 4fold and Mit-suba with 3fold symmetry). These novel proteins are interesting synthetic proteins and serve multiple purposes. Using the Pizza protein we have demonstrated mathematical guided self-assembly according to the lowest common multiplier rule. Furthermore, Pizza-derived proteins are able to biomineralize nano crystals resulting in protein dimerization. Other protein derivatives can be used as building blocks to create fibers, tubes and protein cages. These novel types of protein materials have a huge potential for applications in drug delivery, tissue engineering and biocatalysis.

P-631**The Pizza proteins as building blocks for filament assemblies**

J. P. M. Vrancken, A. R. D. Voet
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We have recently designed a series of self-assembling protein building blocks that are based on the B-propeller architecture. These proteins were named after Pizza. The first developed Pizza protein, Pizza6, contains a six-fold symmetrical architecture formed by six identical tandem repeats of 42 amino acids.

When derivatives of the Pizza6 are expressed with a varying number of repeats they will self-assemble into larger complexes with a predefined stoichiometry. The complexes will fold via simple arithmetical rules into six-bladed structures to regain their six-fold symmetry. Thus Pizza2 and Pizza3 will regain a six-bladed structure while other Pizza proteins will oligomerise to generate a structure of multiple six-bladed structures. Later another Pizza derivative was developed to oligomerise upon the addition of CdCl₂ specifically.

Now, we have combined derivatives of the Pizza proteins with alpha helical coiled coil structures to design large symmetrical filamentous protein complexes. This has been done by using either parallel or anti-parallel coiled-coil fragments fused to Pizza protein derivatives.

Here we present the design, purification and structural and biophysical characterisation of these filament to form novel protein building blocks for different putative applications.

Posters

– 20. Active matter –

P-632 (O-118)

Flagella-mediated unspecific adhesion of *Chlamydomonas* to surfaces is switchable by light

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The natural habitats of many microorganisms such as bacteria and microalgae are liquid-infused confined geometries, e.g. the interstitial space of rocks and soil, where interactions with interfaces are of paramount importance. We performed *in vivo* micropipette force spectroscopy experiments on the unicellular biflagellated microalga *Chlamydomonas*, a prime model organism in cell- and microbiology. We discovered that the flagella-mediated unspecific adhesion to surfaces can be switched on and off by light. Single-cell micropipette experiments show that the light-switchable adhesiveness of the flagella is a completely reversible process that appears to be based on a redistribution of adhesion-promoting flagella membrane proteins on a timescale of seconds. Light-switchable adhesion enables the cell to regulate the transition between planktonic and surface-associated state, which possibly represents an adhesive adaptation to optimize the photosynthetic efficiency in conjunction with phototaxis. The kinetics of the light-induced active approach of the cell towards the surface via flagella-surface contacts is monitored in time-resolved experiments and linked to a model, taking into account the forces generated by the molecular motors under external load.

P-634 (O-120)

Spatial confinement of active microtubule networks induces large-scale rotational cytoplasmic flowK. Suzuki^{1,2}, M. Miyazaki^{1,2}, J. Takagi¹, T. Itabashi^{1,2}, S. Ishiwata¹¹Department of Physics, Faculty of Science and Engineering, Waseda University, Japan; ²Waseda Bioscience Research Institute in Singapore (WABIOS), Singapore

Collective behaviors of motile units through hydrodynamic interactions induce directed fluid flow on a larger length scale than individual units. The motor-driven elongation of microtubule (MT) bundles generates turbulent-like flow in purified systems; however, it remains unclear whether and how MT bundles induce large-scale directed flow like the cytoplasmic streaming observed in cells. Here, we adopted *Xenopus* egg extracts as a model system of cytoplasm and found that MT bundle elongation induces directed flow for which the length scale and timescale depend on the existence of geometrical constraints. At the lower activity of dynein, kinesins bundle and slide MTs, organizing a random network of extensile MT bundles. When the extracts were encapsulated in droplets, the extensile bundles pushed the droplet boundary. This force initiated symmetry breaking of the randomly oriented bundle network, leading to bundles aligning into a rotating vortex structure. This vortex induced rotational cytoplasmic flows on the length scale and timescale that were 10- to 100-fold longer than the vortex flows emerging in bulk extracts. Our results suggest that MT systems use not only hydrodynamic interactions but also mechanical interactions to induce large-scale temporally stable cytoplasmic flow.

P-633 (O-119)

Swimming and rafting of *E. coli* microcolonies at air-liquid interfacesG. Sinibaldi¹, V. Iebba¹, M. Chinappi¹¹Department of Mechanical and Aerospace Engineering, Sapienza University of Rome, via Eudossiana 18, 00184, Rome, Italy; ²Istituto Pasteur Cenci Bolognietti Foundation, Public Health and Infectious Diseases Dept., Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy; ³Department of Industrial Engineering, University of Rome Tor Vergata, Via del Politecnico, Rome 00133, Italy

The dynamics of active colloidal suspension and swimming microorganisms is strongly affected by solid-liquid and air-liquid interfaces. In this contribution, we discuss the motion of *E. coli* at an air-liquid boundary. We experimentally observed and characterized the motion of both single *E. coli* and microcolonies. Both of them follow circular trajectories. Single bacteria preferentially show a counter-clockwise motion, in agreement with hydrodynamics simulation results obtained via a boundary element method (Pimponi et al. J. Fluid Mech. 2016, vol. 789, pp. 514–533). Instead, no preferential rotation direction is observed for microcolonies suggesting that their motion is due to a different physical mechanism. We propose a simple mechanical model where the microcolonies move like rafts constrained to the air-liquid interface to partially explain the experimental data. Finally, we observed that the microcolony growth is due to the aggregation of colliding single-swimmers, suggesting that the microcolony formation resembles a condensation process where the first nucleus originates by the collision between two single swimmers.

P-635 (O-149)

Spontaneous and induced gait-switching in microswimmers

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Self-propulsion by structures known as cilia and flagella presents a significant selective advantage. Great variability exists in the number of flagella, their beating modes, and the basal architecture whence the flagella emanate. In certain bacteria, flagella bundle behind a rod-shaped cell to push the organism forward, while the model alga *C. reinhardtii* uses two near-identical flagella to pull itself through the fluid, executing a breaststroke. In reality, neither gait is stereotypical. For free-living unicellular eukaryotes with few flagella the question of their actuation and coordination has been receiving growing attention from theorists and experimentalists alike. Performing a comparative study across select flagellates, we demonstrate an unprecedented diversity in swimming gaits and reveal the extent to which control of flagellar motility is driven intracellularly. Stochastic bifurcations between different modes of swimming are visualised at high spatiotemporal resolution, and dynamic changes in flagellar beating shown to elicit in trajectory reorientation and responsive navigation at the level of individual cells. These insights suggest that the capacity for fast transduction of signal to peripheral appendages may have evolved far earlier than previously thought.

Posters

– 20. Active matter –

P-636

How growth conditions affect bacterial chemotaxis responses

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The soil bacterium *Pseudomonas putida* propels itself with the help of several flagella that are polarly arranged at one end of the cell body. Through its chemosensory system, which is coupled to the rotary motors that drive the flagella, *P. putida* senses changes in its environment and responds by altering its motility.

When growing in a medium that offers only minimal amounts of nutrient, *P. putida* exhibits a motion pattern characterized by persistent runs that are interrupted by reversals in the swimming direction. In contrast, when growing under rich medium conditions, the reversal rate is drastically reduced and the swimming pattern is dominated by stopping events that interrupt the episodes of persistent runs.

Chemotaxis responses of bacteria are also affected by the medium in which they are grown. When exposed to a uniform increase in chemoattractant concentration, the time evolution of reversal and stop rates for bacteria grown in rich medium does not show any response. However, cells that were grown in minimal medium respond to a sudden increase in chemoattractant by a pronounced change in the reversal rate.

P-638

Pressure of a gas of underdamped active dumbbells

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The pressure exerted on a wall by a gas at equilibrium does not depend on the shape of the confining potential defining the walls. In contrast, it has been shown recently that a gas of overdamped active particles exerts on a wall a force that depends on the confining potential, resulting in a net force on an asymmetric wall between two chambers at equal densities. Here, considering a model of underdamped self-propelled dumbbells in two dimensions, I will discuss how the behavior of the pressure depends on the damping coefficient of the dumbbells, thus exploring inertial effects. In particular, I will show that the force exerted on a moving wall between two chambers at equal density vanishes continuously at low damping coefficient and exhibits a complex dependence on the damping coefficient at low density, when collisions are scarce. I will further show that this behavior of the pressure can to a significant extent be understood in terms of the trajectories of individual particles close to and in contact with the wall. Finally, I will discuss how the notion of pressure is progressively recovered when the active dumbbells are subject to Brownian noise.

Reference :

M. Joyeux and E. Bertin, *Phys. Rev. E* **93** (2016) 032605

P-637

Universality in Incompressible Active FluidsL. Chen¹, J. Toner², C. F. Lee³¹China University of Mining and Technology, China;²University of Oregon, USA; ³Imperial College London, UK

Biology systems operate in the far from equilibrium regime and one defining feature of living organisms is their motility. In the hydrodynamic limit, a system of motile organisms may be viewed as a form of active matter. We have used the concept of universality to categorise some of the emergent behaviour observed in active living matter. Specifically, focusing on a generic model of incompressible active fluids, we have shown that the system's behaviour at the order-disorder phase transition constitutes a novel universality class [1], while that in the ordered phase in 2D belongs to the Kardar-Parisi-Zhang universality class [2].

References:

[1] L. Chen, J. Toner, and C. F. Lee, "Critical phenomenon of the order-disorder transition in incompressible active fluids," *New J. Phys.*, vol. 17, p. 42002, 2015.

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P-639

Consequence of hydrodynamics for bacterial flagellar filaments

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Many cellular organisms possess flagella, slender whiplike appendages which are actuated in a periodic fashion in fluids and allow the cells to self-propel. In particular, most motile bacteria are equipped with multiple helical rotating flagella which interact through the fluid, synchronise, and can form a tight helical bundle behind a swimming cell. We highlight in this talk two consequences of hydrodynamics for bacterial flagellar filaments. First we show how interactions between flagella mediated by the fluid allow them to repeatedly bundle and unbundle leading to reorientation of the whole cell during so-called 'tumble' events. We next show how the flagellar flows induced by bacteria which have differentiated to a swarming state are responsible for large-scale fluid circulation at the scale of the whole swarm.

Posters**– 20. Active matter –****P-640****Pattern formation in microtubule-motor mixtures**I. Maryshev¹, D. Marenduzzo², A. Goryachev¹, A. Morozov²¹School of Biological Sciences, The University of Edinburgh, UK; ²SUPA, School of Physics & Astronomy, The University of Edinburgh, UK

Microtubules (MT) are rod-like biopolymers found in all eukaryotic cells. Spatial distribution of MTs inside the cells is controlled by molecular motors — small molecules that produce directed motion along MT filaments. It is currently believed that MT-motor mixtures are a key ingredient of many large-scale patterns inside cells including mitotic spindles.

This hypothesis is supported by the experiments in the simplified quasi-2D in vitro systems, where interactions between stabilised MTs and motors were demonstrated to lead to spontaneous ordering. The resulting patterns resemble MT distribution observed in living cells thus offering a minimal model system to understand in vivo cytoskeleton dynamics. Here we develop a model to describe pattern formation in MT-motor mixtures. We observe that motors simultaneously associated with two MTs can reorient them with respect to each other. This behaviour is encoded in a kinetic equation that treats motor-mediated interaction between MTs as ‘collisions’. We derive new mean-field equations for the density of MTs, their orientation, and a nematic Q-tensor, and perform the stability analysis and simulate their dynamics numerically. We observe formation of the steady-state stripe-like structures and describe the onset of chaos in such systems.

P-642**Tumbling-to-swimming transition of peritrichously flagellated bacteria**

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Tumbling-to-Swimming transition of Peritrichously Flagellated Bacteria

Peritrichously flagellated bacteria, such as *Escherichia coli*, use rotary motors to rotate helical flagellar filaments. The rotation of each motor is transmitted to a flexible rod called the hook which in turns transmits it to a helical filament, leading to swimming. The motors are randomly distributed over the body of the organism, and thus one expects the propulsive forces from the filament to almost cancel out leading to negligible swimming. Here we show theoretically that the transition to swimming is an elasto-hydrodynamic instability arising when the flexibility of the hook is below a critical threshold. Using past measurements, we demonstrate how the design of real bacteria allows them to be safely on one side of this instability and promotes systematic swimming.

P-641**High-frequency microrheology reveals cytoskeleton dynamics in living cells**

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Living cells are viscoelastic materials, with the elastic response dominating at long timescales ($\gtrsim 1$ ms). At shorter timescales, the dynamics of individual cytoskeleton filaments are expected to emerge, but active microrheology measurements on cells are scarce. Here, we develop high-frequency microrheology (HF-MR) to probe the viscoelastic response of living cells from 1 Hz to 100 kHz. We report the viscoelasticity of different cell types and upon cytoskeletal drug treatments. At previously inaccessible short timescales, cells exhibit rich viscoelastic responses that depend on the state of the cytoskeleton. Benign and malignant cancer cells revealed remarkably different scaling laws at high frequency, providing a univocal mechanical fingerprint. Microrheology over a wide dynamic range up to the frequency of action of the molecular components provides a mechanistic understanding of cell mechanics.

P-643**Balancing assembly and contraction in a reconstituted minimal actin cortex**

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Fast remodeling of the actin cortex is critical for a eukaryotic cell to adapt to its environment. This regulation is brought about by a local fine-tuning of the opposing constructive and destructive processes acting on the cytoskeleton. Here, we report the reconstitution of a minimal model of the dynamic actin cortex to investigate if catalytic polymerization of actin on membranes can coexist with and counterbalance myosin contractility. The Arp2/3-N-Wasp module was employed to assemble actin networks on supported lipid bilayers. Using TIRF microscopy, we show that membrane-associated dendritic actin networks are contracted by muscle Myosin-II filaments. Interestingly, Myosin-II also extracts the fragmented actin from the membrane in a concentration-dependent manner, potentially contributing to actin turnover. The modularity of our synthetic approach enables us to independently tune the strength of formative and disruptive mechanisms, and introduce additional regulators that can shift the equilibrium between them. Through these manipulations, we hope to gain insights into the dynamic interplay of conflicting processes that modulate the actin cytoskeleton.

Posters

– 20. Active matter –

P-644

Mechanical interactions affect biological evolution in bacterial colonies

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Bacterial conglomerates such as biofilms and microcolonies are ubiquitous in nature and play an important role in industry and medicine. In contrast to well-mixed cultures routinely used in microbial research, bacteria in a microcolony interact mechanically with one another and with the substrate to which they are attached. Here we use a computer model of a microbial colony of rod-shaped cells to investigate how physical interactions between cells determine their motion in the colony and how this affects biological evolution. We show that the probability that a faster-growing mutant “surfs” at the colony’s frontier and creates a macroscopic sector depends on physical properties of cells (shape, elasticity, friction). Although all these factors contribute to the surfing probability in seemingly different ways, they all ultimately exhibit their effects by altering the roughness of the expanding frontier of the colony and the orientation of cells. Our predictions are confirmed by experiments in which we measure the surfing probability for colonies of different front roughness. Our results show that physical interactions between bacterial cells play an important role in biological evolution of new traits, and suggest that these interaction may be relevant to processes such as *de novo* evolution of antibiotic resistance.

P-646

Spiral-coil formation in self-propelled chain systemY.-K. Wang¹, C.-C. Liao¹, W.-C. Lo², C.-J. Lo¹¹Department of Physics, National Central University, Taiwan; ²Department of Physics, Duke University, USA

There are rich phenomena in the behaviors of active matters. However, due to experimental technique limitation, only few of the variables can be explored through the experiments. In the previous experiments of two-dimensional bacterial swarm, we observed that the ultra-long bacteria can rotate themselves to form the spiral coils with constant angular speed. At that time, we can simulate the same result in the coarse grained Brownian dynamics simulation of semi-flexible chains. In this report, we aim to further investigate and analyze the mechanism of the spiral coil formation and the interaction between long chain and background short chains. It is easy to generalize that the formation of spiral coil is based on the effective persistence length of long chain. Persistence length is a basic mechanical property quantifying the stiffness of a long chain that is the ration of bending stiffness and thermal energy. In the different density, background short chains play different roles in the spiral-coil formation. At low background density, short chains increase the bending ability of long chain. However, at high density, short chains gather around the long chain, and decrease the spatial configuration of long chain.

P-645

Dynamic and Programmable Self-assembly of Micro-rafts at Air-water InterfaceW. Wang¹, J. Giltinan¹, S. Zakharchenko¹, M. Sitti^{1,2}¹Max Planck Institute for Intelligent Systems, Germany;²Carnegie Mellon University, USA

Dynamic self-assembly consumes energy to maintain spatiotemporal structures and functions. Programmable self-assembly translates information from individuals to the collective whole. Combining dynamic and programmable self-assembly in one platform opens up the possibilities to investigate both types of self-assembly simultaneously and to explore their synergy. This task is challenging because of the difficulty in finding suitable interactions that are both dissipative and programmable. Here we present a dynamic and programmable self-assembling material system consisting of spinning at air-water interface circular magnetic micro-rafts of radius 50 μm and with cosinusoidal edge-height profiles. The cosinusoidal edge-height profiles not only create a net dissipative capillary repulsion that is sustained by continuous torque input, but also enables directional assembly of micro-rafts. We uncover the layered arrangement in the patterns formed by dynamic self-assembly and offer mechanistic insights. We demonstrate programmable self-assembly and show that a 4-fold rotational symmetry encoded in individual micro-rafts translates into 90° bending angles in the assembled structures. Our material system can serve as a model for studying non-equilibrium dynamics and statistical mechanics.

P-647

FeTPPS, not just a peroxynitrite decomposition catalyst

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5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrinato iron (III) chloride (FeTPPS) is an artificial water soluble derivative of heme, which has the same porphyrin ring and the same active site. FeTPPS has been used as a scavenger of peroxynitrite for inhibiting ONOO⁻ from causing nitration of tyrosine residues and antioxidant protects against oxidative injuries in many studies. However, my studies indicate that FeTPPS is a pseudo-peroxidase, like heme, and can cause protein tyrosine nitration by the way of peroxidase-H₂O₂-NO₂⁻ in *vivo* and *vitro*. Interestingly, FeTPPS may on the one hand, eliminates ONOO⁻ induced tyrosine nitration, but on the other hand, catalyzes protein tyrosine nitration when H₂O₂ and NaNO₂ are presented. Protein tyrosine nitration was considered as the footprint of peroxynitrite. Taken together, FeTPPS is not an appropriate agent to test in *vivo* presence of ONOO⁻ or not. Besides, the property of hydrophilic selectivity of FeTPPS may be used as a site selective nitrating agent for the functional study of specific tyrosine residues nitration in protein.

Posters*– 20. Active matter –***P-648****Hydrodynamics of collectively migrating cellular fluids**

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Collective cell migration plays a notable role during wound healing, embryonic development and cancer progression. On long time scales, collectively migrating cells can be reminiscent of a fluid flow. Using microstructuring techniques to confine cells to geometries akin to those found in classical fluid dynamics experiments, we investigated the hydrodynamics of active cellular matter.

In analogy to laminar flow of a Newtonian fluid through a pipe, we studied the invasion of MDCK cells into straight channels. The underlying flow field was extracted by particle image velocimetry, uncovering a plug-like flow across the channel. Using the Fisher-Kolmogorov model, we derived the collective diffusion coefficient of the density-gradient dependent diffusive-like contribution to collective migration. In order to connect diffusion mediated transport to underlying cellular motility, single cell trajectories and the occurrence of vortices were studied.

Further classical hydrodynamic problems can be investigated, such as the migration of cells through a nozzle. We intend to use the experimental results for comparison with and validation of a theoretical model for active nematic-isotropic mixtures.

Posters

– 21. Membrane permeation: transporters –

P-649 (O-124)

Using bacteria to fight bacteria: Parasitisation of ferredoxin-uptake receptors in *Pectobacterium*C. Thompson¹, R. Grinter², D. Walker¹, O. Byron¹¹University of Glasgow, UK; ²Monash University, Australia

Antibiotic resistance is a global problem affecting both health and food security. Gram-negative plant pathogens, such as *Pectobacterium*, have detrimental effects on crop yields. Bacteriocins are protein antibiotics that are usually active only against bacteria closely related to the producing strain; these bacteriocins are often species specific and therefore offer an alternative to the current antibiotics used in clinical practice. Three recently described bacteriocins produced by *P. carotovorum* consist of a [2Fe-2S] plant-type ferredoxin domain fused to a bacteriocin cytotoxic domain. These parasitize an existing ferredoxin uptake system to gain entry into target cells. The normal physiological role of this uptake system is to acquire iron from ferredoxin. We have identified the ferredoxin/pectocin receptor as a TonB-dependent receptor that we have designated FusA. Bioinformatic analysis indicates the existence of a ferredoxin uptake operon, which in addition to the receptor FusA, encodes a protease, an ABC-transporter which likely act to cleave ferredoxin and transport liberated iron to the cytoplasm and a homologue of TonB. We are currently dissecting the roles of these proteins in ferredoxin uptake using a combination of structural (SANS and SAXS) and functional studies.

P-651 (O-126)

An emerging technique for the characterization of transport proteins: SSM-based electrophysiologyA. Bazzone, M. Barthmes, A. Brüggemann, N. Fertig
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In the past 10 years, Solid Supported Membrane (SSM)-based electrophysiology has been proven as an efficient tool for the characterization of electrogenic membrane proteins, e.g. transporters, ion pumps and ion channels. It's a label-free, high-sensitivity method which allows the use of membrane preparations, e.g. reconstituted protein samples and membranes from native tissues or cell culture. The high sensor stability allows for different solution exchange experiments using the same sensor. Both transport and binding can be resolved and kinetic parameters like rate constants, KD, KM or IC50 can be determined in a fast and easy workflow. Until now more than 100 different proteins have been tested; almost 100 peer reviewed papers have been published. Here we show data for several targets revealing different aspects of their transport mechanism. The organic cation transporter OCT2 transports multiple substrates with different EC50 and Vmax. The proton-coupled peptide transporter PEPT1 was used to show an inhibition assay. We analyzed different transport modes of the Na⁺/Ca²⁺ exchanger NCX and assayed binding and transport reactions of different sugar transporters individually. Moreover results for the Na⁺/K⁺-ATPase and the nicotinic acetylcholine receptor (nAChR) are shown.

P-650 (O-125)

Membrane protein diffusion in living *E. coli*: from fundamentals to insight in protein translocation

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The organization of the bacterial membrane has been challenging to analyze due to the small size and non-flat geometry of bacterial cells. We have used single-molecule fluorescence microscopy and three-dimensional quantitative analyses in live *Escherichia coli* to demonstrate that its cytoplasmic membrane contains microdomains with distinct physical properties. We showed that the stability of these domains depends on the integrity of the MreB cytoskeletal network. Cytoskeleton and membrane affect trans-membrane protein (TMP) diffusion. The mobility of the TMPs tested is subdiffusive, most likely caused by confinement of TMP mobility by the submembranous MreB network.

We then aimed to understand how the twin-arginine translocation (Tat) system transports fully folded proteins across the cytoplasmic membrane of bacteria. The pore-forming subunit TatA reversibly associates with substrate-binding TatBC complexes. We tracked individual eGFP-fused TatA complexes and showed that large, stable TatA complexes switch between fast and slow diffusion, with diffusion coefficients ~10-fold different. Mechanistic consequences are discussed.

P-652

Conformational dynamics of the Na⁺/H⁺ antiporter studied by EPR spectroscopyS. Dunkel¹, E. Padan², G. Masrati³, N. Ben Tal³, J. P. Klare¹, H.-J. Steinhoff¹¹Physics Department, University of Osnabrück, Germany;²Structural Biology, Molecular Biology, Hebrew University of Jerusalem, Israel; ³Department of Biochemistry and Molecular Biology, Tel Aviv University, Israel

The Na⁺/H⁺ antiporter NhaA, a dimeric integral membrane protein of *Escherichia coli*, belongs to the class of monovalent cation proton antiporters of the so called CPA superfamily. The main role of NhaA is the regulation of cytoplasmic pH and sodium content by the pH-regulated coupled transport of 2 H⁺ and 1 Na⁺ in a 2:1. The protein comprises two domains – the dimer and the core domain. The different conformational states of the transport cycle are not fully understood, but mechanistic models have been proposed, describing the motion of the core domain during the transport cycle as either a rocking bundle or an elevator mechanism.

We studied conformational changes of NhaA induced by pH and upon substrate binding by site-directed spin labeling and EPR spectroscopy to get insights into the transport mechanism. Comparison of the available crystal structures (inward-open) and of homology models for the outward-open conformation with our experimental results reveals conformational changes upon substrate binding and pH change. We observe multiple conformational states sampled under all conditions tested, indicating conformational heterogeneity, similar to what has been found for the sodium-coupled Asp transporter Glt_{pH}.

Posters

– 21. Membrane permeation: transporters –

P-653

Is it possible to reduce TFP concentration required for MDR reversal and apoptosis induction?

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Overexpression of P-glycoprotein (Pgp) encoded by the MDR1 gene is one of the major obstacles to successful cancer chemotherapy. The discovery that trifluoroperazine (TFP) is a competitive inhibitor of Pgp transport activity that can contribute to the sensitization of resistant cancer cells to chemotherapy triggered research to concentrate on chemosensitizers. Unfortunately, TFP itself cannot be used as a chemosensitizer in patients due to the necessity of use of high concentrations that are intolerated by humans due to unbearable side effects. The goal of this study was to evaluate if newly-synthesized oxycam derivatives enhanced cytotoxicity of TFP in MDR1-transfected Madin-Darby canine kidney (MDCK-MDR1) cells. Two of five studied oxycam derivatives allowed for the significant reduction of TFP concentration required for MDR reversal, apoptosis induction, microfilament organization change, and cellular lipid peroxidation.

P-655

The pH dependence of synthetic anion transporters

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Synthetic transmembrane anion transporters, named anionophores, could correct the lack of anion transport in cystic fibrosis (CF) patients, independently of the CFTR mutation, being a promising therapy for this genetic disease. Prodigiosin and its derivatives have been proposed as immunosuppressive, antibiotic, antimalarial and anti-tumoral drugs. We studied the properties of prodigiosine inspired molecules with reduced toxicity and high transport capacity. These anionophores exchange anions across the lipid bilayer. Interestingly, some of these molecules are more active at acidic pH than at basic pH values. Measurements of Cl[−] efflux in vesicles at various pH conditions, and different pH gradients, show that these anionophores hardly co-transport H⁺ (or OH[−]), as previously suggested. We propose that the anionophore is in equilibrium between states neutral, ionized and anionic-complexed. Thus, pH determine the amount of the anionic-complexed form, that ought be the form that exerts the anion transport. We conclude that optimal lead compounds for the CF therapy should be selected among those with a pK_a value higher than the working pH. *The project TAT-CF has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 667079*

P-654

Characterization of a PIB-ATPase from the psychrophilic bacteria *Bizionia argentinensis*

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PIB-ATPases are integral membrane proteins which are widespread distributed in nature, and actively transport heavy metal ions through cell membranes coupled to the hydrolysis of ATP. In this work we isolate and characterize a putative PIB-type ATPase from the Antarctic bacterium *Bizionia argentinensis* (BaCopA). This protein was cloned and expressed in *S. cerevisiae* as a GFP-fusion protein. Membrane proteins were solubilized with sodium dodecyl sulfate and BaCopA purified by IMAC chromatography. The obtained protein run as a single band on SDS PAGE, and display ATPase and phosphatase activity with a maximum around 20°C and pH 8.0. The ATPase activity is enhanced by Mg²⁺ and phospholipids, and inhibited by the Cu(I) chelator BCS. An homology structural model was obtained using the resolved structure of *L. pneumophila* CopA as template (PDB: RFU3). The structural alignment show a high degree of similarity, with the typical topological pattern of P_{1B}-1 ATPases: an N-terminal Heavy Metal Associated domain (residues 4-62), an actuator domain (residues 296-509), an ATP binding domain (residues 522-741), and a transmembrane domain composed of 8 transmembrane helices including the characteristic residues involved in Cu(I) binding and translocation.

With grants from UBA, CONICET and ANPCyT.

P-656

Intra-membrane protein hydration: the role of lateral pressure in a metal-transporting ATPase

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Ion transport across biological membranes requires ion hydration shells to interact with the interior of membrane proteins. Lipid protein interactions affect metal transport through P-type ATPases, but the influence of lipids on internal dielectrics is hard to measure. We have site-specifically labeled internal cysteines of the P_{1B}-type copper ATPase CopA (*Legionella pneumophila*) with the solvatochromic dye BADAN. This enabled dipolar relaxation studies in a solubilized form of the protein and in a native lipid environment provided by nanodiscs (NDs). Time-dependent (ns) fluorescence shifts of BADAN bound to either of the two active site cysteines, Cys382 and Cys384, revealed the local hydration and dipole mobility. At Cys382, both parameters were strongly reduced upon lipid insertion of the ATPase. The environment of Cys384, although less than a helical turn apart, was less affected and, against expectation, dipole mobility increased in NDs as compared to the solubilized state. Thus, hydration and dipole mobility are shaped significantly and independently of each other by membrane lateral pressure. Strain in the lipidic phase generated by conformational changes of the ATPase may thus contribute to restoring hydration patterns during cyclic protein function.

Posters

– 21. Membrane permeation: transporters –

P-657

Identification of the pK value of Glu325 in Lactose permease by SEIRAS-perfusion approachN. Grytsyk¹, J. Sugihara^{2,3}, R. Kaback^{2,4}, P. Hellwig¹

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The major facilitator superfamily (MFS) is a large and diverse family of membrane proteins that transport substrates ranging from ions to peptides and drug molecules. The translocation process occurs by an alternating access mechanism involving transitions between inward-facing and outward-facing conformations. The pKa of Glu325 in the lactose permease is very alkaline, and protonation of this residue is essential for effective binding of galactopyranosides. Thus, Glu325 plays a central role in “coupling.”

Here, we present a coupled perfusion induced and surface enhanced infrared spectroscopy (SEIRAS) approach to study the role of Glu325 in LacY in the presence and absence of a sugar analog (Nitrophenyl- α -D-Galactopyranoside). Infrared difference spectra reveal signals characteristic for conformational changes and protonated acidic residues. Wild type LacY and different mutants, including, E325A, G46W/G262W (LacYww) and LacYww/E325A, have been studied.

Experimental evidence is given that Glu325 has a pKa of 10.5 ± 0.1 , a value that coincides precisely with the variation of the affinity of LacY for galactoside as a function of pH. The conclusion provides strong confirmation for the critical role of this residue.

P-659

Uncoupling proteins are highly sensitive to the membrane lipid compositionO. Jovanovic¹, K. Chekashkina², P. Bashkurov², M. Vazdar³, E. E. Pohl¹

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Lipid composition is important for the function of membrane proteins. We studied the effect of lipids with different shapes on the activity of the mitochondrial uncoupling protein 1 (UCP1). We focused (i) on native lipids with positive or negative curvature, such as lyso 1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine, various phosphoinositides (PIP5, PIP35, PIP345) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and (ii) on DOPE modified by reactive aldehyde 4-hydroxy-2-nonenal and 4-oxo-2-nonenal (HNE-DOPE, ONE-DOPE – adducts¹). By combining the measurements of the total membrane conductance², bending moduli of the lipid bilayer and performing MD simulations, we have revealed that (i) positively shaped lipids increase UCP1 activity, (ii) HNE-DOPE and ONE-DOPE – adducts act similar to positively shaped lipids, and (iii) negatively shaped DOPE does not influence the studied protein. We propose that mitochondrial proteins are only regulated by lipids with positive curvature which influence membrane mechanical properties.

References: 1. Jovanovic O. et al. Free Radical Biology and Medicine, T. 89. pp. 1067-1076, (2015) 2. Beck V. et al. BBA Bioenergetics, V 1757 (5-6) pp. 474-479, (2006)

P-658

Molecular simulations of cardiolipin interactions with the adenine nucleotide translocaseG. Hedger¹, S. L. Rouse^{1,2}, J. Domański¹, M. Chavent¹, H. Koldsø^{1,3}, M. S. P. Sansom¹

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The exchange of ADP and ATP across the inner mitochondrial membrane is a fundamental cellular process. This exchange is facilitated by the adenine nucleotide translocase, the structure and function of which are critically dependent on the signature phospholipid of mitochondria, cardiolipin (CL). Here¹ we employ multiscale molecular dynamics simulations to investigate CL interactions within a membrane environment. Using simulations at both coarse-grained and atomistic resolutions, we identify three CL binding sites, in agreement with those seen in crystal structures and inferred from nuclear magnetic resonance measurements. Characterization of the free energy landscape for lateral lipid interaction via potential of mean force calculations demonstrates the strength of interaction compared to those of binding sites on other mitochondrial membrane proteins, as well as their selectivity for CL over other phospholipids. Extending the analysis to other members of the family suggests a degree of conservation. Simulation of large patches of a model mitochondrial membrane containing multiple copies of the translocase show that CL interactions persist, and suggests CL may mediate interactions between translocases.

1. Hedger et al., 2016, Biochemistry, 55:6238-6249

P-660

Transport of bacterial lipopolysaccharides to liposomes and immune cell membranesF. Kopp¹, S. Kupsch¹, L. Paulowski^{1,2}, U. Mamat³, T. Gutschmann², A. B. Schromm¹

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Recognition of bacterial lipopolysaccharide (LPS) is a key event in innate immune responses to infections. The activation of intracellular receptors inducing pyroptosis requires uptake and cytosolic localisation of LPS. Mechanistic insights how LPS is delivered to intracellular sites are scarce. Using DOPC and phase-separated DOPC/SM/CHOL membranes we have investigated the possibility of transport of LPS to membranes by the Lipopolysaccharide-binding protein (LBP). We characterized LBP membrane interaction by FACS, FRET-spectroscopy, and CLSM. We observed membrane interaction of LBP with small, large and giant liposomes with broad specificity. Transport of LPS to liposome membranes was addressed by a FRET-assay. These experiments show a transport capacity of LBP delivering LPS into host cell mimicking membranes. In biological studies on HEK293 cells and human macrophages we investigated LBP binding, uptake and intracellular transport of LPS. The demonstration of LPS transport to reconstituted membranes can provide a mechanistic basis for the role of LBP in intracellular LPS-trafficking and signalling.

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Posters

– 21. Membrane permeation: transporters –

P-661

Elucidation of ion selectivity of NaR by electrophysiological measurementY. Kozaki¹, S. Hososhima¹, S. Tsunoda^{1,2}, H. Kandori¹¹Nagoya Inst. Tech., Japan; ²JST•PRESTO, Japan

The microbial rhodopsins are a class of membrane proteins with seven transmembrane helices harboring an all-*trans* retinal which is covalently bound to a side chain of a lysine residue via Schiff base. They are widely distributed through eukaryotic and prokaryotic organisms and exhibited diverse, specialized functions. Their molecular activity involves active ion transport, passive ion conductance, a light sensation which initiates signal transduction for phototaxis responses, and several enzymatic activities such as histidine-kinase, cyclase and phosphodiesterase.

Sodium pumping rhodopsins (NaRs) actively transport Na⁺ and H⁺ depending on the ionic condition, i.e. NaR transports Na⁺ in the presence of Na⁺ in the solution, and pumps H⁺ in the absence of Na⁺.

Our attempts to gain a more precise understanding of the biophysical properties of NaRs, we here performed electrophysiological measurements on FdNaR, from *Flagellimonas sp. DIK*.

The light-induced pumping current was observed when FdNaR was expressed in ND7/23 cells. We then systematically changed the ionic conditions of the intercellular-side and the extracellular side independently. The results suggest that the FdNaR probably transports both Na⁺ and H⁺ under the physiological condition.

P-663

Molecular mechanisms of proton transfer in the protein secretion motor SecDFT. Mori¹, T. Tsukazaki², Y. Sugita¹¹RIKEN, Japan; ²Nara Inst. Sci. Tech., Japan

About 30% of proteins are secreted across membranes or integrated into membranes. The Sec translocon plays a central role in these protein translocations. It consists of protein complexes: SecA ATPase, protein channel SecYEG, and membrane chaperone SecDF. Recently, it has been revealed that SecDF undergoes large conformational change between F- and I-forms by the proton-motive force, where the conserved Asp in the transmembrane region is important for proton transfer [1]. However, their relationships between conformational change and proton transfer has not been understood well. To elucidate their molecular mechanisms, we carried out all-atom molecular dynamics simulations of SecDF in explicit membranes. We found that the protonation state of the conserved Asp affects the conformational change of SecDF as well as water channel formation in the transmembrane region. We discuss detailed mechanisms for the function of SecDF.

[1] Tsukazaki et al., Nature, 474, 235–238 (2011).

P-662

Genipin lacks specificity for UCP2J. Kreiter¹, A. Rupprecht¹, L. Zimmermann¹, M. Fedorova², M. Moschinger¹, L. Gille¹, E. E. Pohl¹¹University of Veterinary Medicine, Vienna, Austria; ²Center of Biotechnology-Biomedicine, University of Leipzig, Germany

Uncoupling proteins (UCPs) are members of the mitochondrial anion-carrier superfamily, which are proposed to regulate proton leak through the inner mitochondrial membrane. Until now, only purine nucleotides have been accepted as an inhibitor of UCP activity. Genipin is a natural cross-linking compound found in *Gardenia jasminoides Ellis*, which has been suggested to be a specific inhibitor of UCP2 activity¹. To test this, we performed electrophysiological experiments on artificial bilayers with recombinant proteins², light microscopy and mass spectrometry. Our results show that genipin inhibits also UCP1, 3 and complex III. We revealed that UCPs inhibition by genipin is similar to the inhibition by purine nucleotides and excluded cross-linking as a molecular mechanism.

1. Zhang et al. (2006), Cell Metabolism, Volume 3, 417–427
2. Beck et al. (2006), BBA - Bioenergetics, Volume 1757, 474–479

P-664

Rat aquaporin-5 is pH-gated induced by phosphorylationA. F. Móscica^{1,2}, C. Rodrigues^{1,2}, A. P. Martins^{1,2}, C. Prista³, G. Soveral^{1,2}

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Aquaporin-5 (AQP5) is a membrane water channel widely distributed in human tissues that was found up-regulated in different tumors and considered implicated in carcinogenesis in different organs and systems. It is known that AQP5 expression and trafficking can be regulated by phosphorylation, but whether phosphorylation also regulates channel activity and contributes to gating still remains uncertain.

In this work, we expressed rat AQP5 in yeast and investigated mechanisms of gating by pH and phosphorylation. We observed that AQP5 does not change its activity by external acidification, but phosphorylation makes the AQP5 channel prone to pH sensing, with higher activity at physiological pH 7.4. Since phosphorylation occurs intracellularly, one may speculate that it is the direct AQP5-phosphorylation that alters protein conformation and, in this new conformation, channel widening results from deprotonation of residues at pH 7.4.

Posters

– 21. Membrane permeation: transporters –

P-665

Glucose concentrations influence on activities of FoF₁ ATPase and hydrogenase 4 in *Escherichia coli*

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Oxidation-reduction potential drop followed medium acidification and biomass formation of *Escherichia coli* wild type BW25113 and hydrogenase 4 (Hyd-4) JRG3621 (*hyfB-R*) mutant were observed upon 0.2% and 0.8% glucose concentrations, pH 5.5–7.5. H₂ formation was inhibited in JRG3621(*hyfB-R*) mutant with the lack of functional Hyd-4 at pH 7.5 upon 0.2% glucose fermentation. 0.8% glucose had 1.3 fold stimulatory effect on wild type and mutant biomass formation. However, mutant growth was inhibited 1.3 fold at pH 7.5 and 2 fold at pH 5.5 compared with wild type. Compared to 0.2, 0.8 % glucose had no stimulatory effect on H₂ formation during wild type growth; and H₂ production rate was inhibited ~5 fold in mutant when 0.2% glucose was supplemented at pH 7.5. Proton efflux via FoF₁ATPase of mutant strain was inhibited upon bacterial growth on 0.2 % glucose at pH 7.5. Moreover, FoF₁ATPase activity of 0.2 % glucose fermented *hyfB-R* mutant was lower 1.5-fold compared to wild type in K⁺-free medium, and slightly increased by K⁺ addition.

The results point out the important role of Hyd-4 for bacterial growth particularly at acidic pH; glucose concentration and pH regulate Hyd-4 activity. Moreover, FoF₁-ATPase's interaction with Hyd-4 upon 0.2% glucose fermentation at pH 7.5 was stated.

P-666

The diverse sensitivity of parental and resistant breast cancer cells after electrochemotherapeuticN. Rembiałkowska¹, A. Choromańska¹, M. Dubińska-Magiera², J. Rossowska³, M. Kotulska⁴, J. Sączko¹, L. M. Mir⁵, J. Kulbacka¹¹Medical Univ., Dept. of Medical Biochemistry, Wrocław, Poland;²Animal Developmental Biology Dept., Institute of Experimental Biology, University of Wrocław, Poland; ³Polish Academy of Sciences, Inst. of Immunology and Experimental Therapy Rudolfa Weigla, Wrocław, Poland; ⁴Univ. of Technology, Institute of Biomedical Engineering and Instrumentation, Wrocław, Poland; ⁵Lab. of Vectorology and Anticancer Therapies, CNRS UMR8203, Univ. P-Sud, Univ. Paris-Saclay, Gustave Roussy, Villejuif, France

Electroporation (EP) is a biophysical method used to increase the uptake of molecules by permeabilization of the cell membrane. The combination of electroporation and drugs with inhibited or limited transport is known as electrochemotherapy (ECT).

We investigated human breast adenocarcinoma (MCF-7/WT) and its doxorubicin resistant subline (MCF-7/DOX). The concentrations of bleomycin were 30nM and 300nM and for doxorubicin 1.7μM. The EP parameters used were: 5 and 8 pulses of 50μs or 100μs duration delivered at a repetition frequency of 1 Hz, with an electric field intensity 100÷2000V/cm. We evaluated the impact of ECT on P-gp and BCRP expression. P-gp and BCRP protein were also determined following exposure to Elacridar (MDR modulator). The efficiency of ECT was evaluated by a viability assay. Apoptosis and necrosis detection was determined by FACS. The cellular ultrastructure was detected and membrane permeabilization was visualized using a membrane marker.

Our results indicate the most pronounced effect of EP combined with drugs in resistant cells in the presence of DOX, with a perturbation of the ultrastructure and a decrease of P-gp expression resistant cells. Sensitive cells occurred less susceptible.

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Posters

– 22. Imaging the cell –

P-667 (O-130)

Application of indirect optical micromanipulation in fluorescent 3D live cell imaging

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Optical micromanipulation of live cells has been extensively used to study cellular phenomena in basic research or in diagnostics frequently coupled with advanced imaging techniques. Photodamage, often associated with direct cell trapping can be reduced with the careful choice of the laser wavelength. Alternatively, using cell-attached intermediate objects as handles could practically eliminate the irradiation damage and increase trapping efficiency due to the high refractive index contrast. Here we introduce the technique of indirect optical micromanipulation using shape-optimized intermediate objects made by two-photon polymerization and enabled by holographic optical tweezers. With this technique the cell-to-focal spot distance is increased to several micrometers while maintaining the cells rapid maneuverability with 6 degrees of freedom. We demonstrate the power of the method with 3D fluorescent live cell imaging. We achieved 3D reconstruction of single cells with isotropic resolution by imaging them at different orientations. The various views of the cells were achieved by rotating them around an axis perpendicular to the optical axis with the optical tweezers. The presented tool and manipulation scheme can be readily applied in a range of optical microscopic techniques.

P-669 (O-132)

Pair correlation analysis of fixed PALM and live PALM applied on the water channel AQP3

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Water transport across the plasma membrane is mediated by aquaporin (AQP) water channels. We previously found that a short-term increase in cAMP leads to an increase in lateral diffusion of AQP3, revealing short-term regulation. To further study if AQP3 is regulated at the nanoscale, we applied pair correlation in combination with PALM. This showed that AQP3 organize in nano-domains smaller than 60 nm and upon cAMP stimulation, changed organization to 60 – 200 nm sized nano-domains. Thus, PC-PALM revealed regulation at the nanometer resolution. Furthermore, we performed live-PALM of AQP3 upon cAMP stimulation. Power-spectral analysis of the single-molecule trajectories revealed that the molecules were not freely diffusing. Rather, data were consistent with a simple model for 2D diffusion in confinement. While the measured diffusion coefficients of AQP3 were identical between control and cAMP stimulated cells, the confinement radius increased significantly. Thus fixed and live PALM measurements both revealed a change of AQP3 nano-organization in the plasma membrane upon cAMP stimulation, indicating short-term hormone regulation of AQP3 at the nanoscale level which has so far been undetectable.

P-668 (O-131)

Transcription factor clusters regulate gene expression in yeast *Saccharomyces cerevisiae*

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In yeast *S. cerevisiae* expression of genes essential for alternative carbon sources metabolism is regulated by the transcription factor (TF) Mig1. Upon glucose limitation, Mig1 becomes phosphorylated and relocates to the cytoplasm. Glucose causes Mig1 dephosphorylation and import to the nucleus where it binds to target promoters mediating gene repression. However, the exact mechanism of gene regulation by Mig1 is unclear. We combined live-cell single-molecule imaging with traditional biochemical methods to study Mig1 localisation and phosphorylation dynamics. Our data show that Mig1 is present as monomer and oligomers in the cytoplasm and nucleus, constantly shuttling between these compartments regardless of glucose presence. We found that Mig1 phosphorylation is not solely regulated by glucose and does not drive Mig1 translocation across the nuclear membrane. Mapping Mig1 target promoter sequences onto a 3D yeast chromosomes structure model allowed us to model the 3D arrangement of Mig1 in the nucleus and was consistent with Mig1 clusters. Studies on another TF showed similar oligomeric organisation. We suggest that yeast gene regulation is mediated via TFs which act as multimeric clusters.

P-670

¹O₂ induced deprivation of extracellular polymeric substances in *Synechocystis* and *Symbiodinium*

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Micro-algal cells do have extracellular polymeric substances (EPSs) layer which covers the surface of the cells and provides a supporting structure for biofilm formation. Singlet oxygen (¹O₂) has been implicated as an important mediator of light induced damage of the photosynthetic apparatus in cells of photosynthetic organisms. It has recently been observed that unicellular microalgae excrete so far unidentified metabolites, which sensitize ¹O₂ outside the cells. However, their mechanism(s) of secretion and physiological role remain(s) unknown. We have applied scanning electron microscopy technique together with light and confocal laser-scanning microscopy techniques in cultured *Symbiodinium* and *Synechocystis* cells to understand ¹O₂ dependent damage of EPSs under light stress condition by supplying external ¹O₂ sensitizer. Our results show light induced damage of EPSs in cultured *Symbiodinium* and *Synechocystis* cells which may be connected to oxidative stress mediated damage. We will present the characterization of the effect of external ¹O₂ by using advanced microscopic imaging techniques and its involvement in initiating the damage of EPSs in intact *Symbiodinium* cells and *Synechocystis* cells.

Posters

– 22. Imaging the cell –

P-671

The effect of CDP-choline on autophagy and mitochondrial dynamics in beta-amyloid treated PC12 cellsB. Bilge¹, H. Gezmis², S. Bozkurt¹, D. Yucel¹, B. Kan¹, I. H. Ulus¹, D. Oz-Arslan¹¹Acibadem University, School of Medicine, Istanbul, Turkey;²Yeditepe University, Istanbul, Turkey

Recent evidence suggests that autophagy may have a crucial role in Alzheimer's disease (AD). Cytidine-5'-diphosphocholine (CDP-Ch), an intermediate in the biosynthesis of membrane phospholipids, is known to have neuro-protective effects but the mechanism remains unclear. In this study, we investigated the effect of CDP-Ch on the autophagy and mitochondrial dynamics during amyloid-beta (A β 1-42) mediated neuronal injury. PC12 cells were differentiated with nerve growth factor (NGF) and treated with amyloid-beta in the presence and absence of CDP-Ch. We followed autophagy induction by LC3-II/I ratio, p62, Atg5, Beclin-1 and PINK1 levels by western blot analysis. Differentiated cells showed an increase in LC3II/I ratio. We also observed that treatment of PC12 cells with A β 1-42 induced autophagy and led to changes in mitochondrial membrane potential. Our preliminary data indicated that the presence of CDP-Ch alters mitochondrial dynamics and autophagic machinery during beta amyloid injury. We are currently studying the effects of CDP-Ch and amyloid beta treatments on mitochondrial respiration and mitochondrial morphology during autophagy induction. This work was supported by The Scientific and Technological Research Council of Turkey (Grant number:114Z494).

P-673

High resolution atomic force microscopy of living *S. aureus* bacterial cell wallJ. M. Burns, R. G. Bailey, S. Kumar, R. D. Turner, N. Mullin, S. J. Foster, J. K. Hobbs
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The aim of the project is to use the high resolution AFM to image living bacterial surfaces, over the course of their life cycle, to measure changes in the architecture of the peptidoglycan chains that make up the bacterial cell wall. *S. aureus* is a Gram positive bacterium with a ~25 nm thick cell wall which maintains the spherical shape of the cells. This biopolymer is restructured over the cell division cycle. The bacteria are immobilised using a silicon wafer etched with holes allowing them to be imaged repeatedly. High resolution imaging is achieved using tapping mode with a small cantilever AFM and through buffer optimisation. High resolution images of *S. aureus* cells were taken at molecular resolution on living bacteria at multiple stages of the cell division cycle. Different structures are observed for different regions of the cell wall, depending on the age, with a transformation from tightly spaced concentric rings to a loose mesh. Quantitative analysis of the images at a molecular scale has been used to determine the concentricity of the rings and bearing analysis on the mesh region gives the depth distribution of the holes showing that some of the holes are as deep as the whole cell wall layer opening questions about how material passes through the cell wall.

P-672

The population growth dynamics of *E. coli* in the presence of cell wall targeting antibiotics

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Cell wall targeting antibiotics are some of the most widely used drugs for the treatment of bacterial infections. There is now concern about the emergence and spread of bacteria resistant to these antibiotics. Efforts to prevent this emergence of resistance are hindered by our lack of understanding of antibiotic-bacteria interactions when designing treatment plans. This project aims to uncover the details of these antibiotic-bacteria interactions for three cell wall targeting antibiotics: cefotaxime, fosfomycin and mecillinam. This will be achieved by combining experimental observations of the growth dynamics and cell morphology, with the predictions of a mathematical model describing the competition between cell wall precursor production and cell wall growth.

P-674

Evaluation of cell malignancy using Digital Holographic MicroscopyV. L. Calin¹, M. Mihailescu², E. Scarlat², A. V. Baluta², E. Kovacs¹, T. Savopol¹, M. G. Moisescu¹
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Early cancer diagnosis with high accuracy represents a major issue in biomedical research. There is a need of finding cancer cell biomarkers by identifying cellular characteristics which may differentiate malignant from normal cells.

Cell refractive index (RI) was proved as cancer biomarker of great potential, being correlated with the cell morphology, division rate or membrane permeability.

We used Digital Holographic Microscopy to compare RI and other optical characteristics of F1 and F10 sublines of B16 murine melanoma cells. The reconstructed quantitative phase images (QPIs) were computed and statistical methods were used for analyzing the distribution of phase shifts within the cells. Results were correlated with the malignancy degree of the F1 and F10 sublines as measured by real time impedance based-assay (RTCA, Roche) and clonogenic tests. The more malignant F10 subline showed higher RI (1.3908-1.4181) and unimodal QPIs histograms, while the less malignant F1 subline presented RI between 1.3546-1.3653 and bimodal histograms. The higher malignancy of F10 cells was confirmed by RTCA and clonogenic tests (higher proliferation rate and bigger colonies).

The RI and QPIs histograms may be thus developed as optical biomarkers for label-free cell malignancy detection.

Posters

– 22. Imaging the cell –

P-675

Cellular imaging of small RNAs using fluorescent RNA-Mango based aptamersA. Cawte^{1,2}, S. Jeng³, A. Autour⁴, A. Abdolazadeh³, A. Galli³, M. Ryckelynck⁴, P. Unrau³, D. Rueda^{1,2}¹Section of Virology, Department of Medicine, Imperial College, UK; ²MRC London Institute of Medical Science, UK; ³Department of Molecular Biology, Simon Fraser University, Canada; ⁴Architecture et Réactivité de l'ARN, CNRS, Université de Strasbourg, France

In recent years, there has been an explosion of SELEX-evolved fluorescent RNA aptamers, such as Spinach and Mango. Fluorogenic RNA aptamers have sparked a lot of interest and hold great potential to enable the visualisation of RNA molecules in cells. However, their applicability has been limited primarily to bacterial cells. Evolving new RNA aptamers with improved physico-chemical properties should better their use in cellular imaging. Three new Mango-based aptamers have recently been evolved using microfluidic-assisted *in vitro* compartmentalization, mutagenesis and fluorescent selection, which improved fluorescence brightness, ligand binding affinity and thermal stability. We show that these aptamers are readily useable to image small non-coding RNAs (such as 5S rRNA and U6 snRNA) in both live and fixed human cells with improved sensitivity and resolution. The imaging data show that the modified RNAs sub-cellular localisation pattern is conserved, as validated using immunofluorescence. Our data show that these new aptamers are vastly improved for cellular imaging over previous variants, and can in principle be incorporated into a wide range of coding and non-coding RNAs.

P-677

Topography of cells revealed by variable-angle Total Internal Reflection Fluorescence microscopyD. El Arawi, M. Cardoso dos Santos, C. Vézy, R. Jaffiol
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Total Internal Reflection Fluorescence (TIRF) microscopy is a widespread technique used to study cellular process occurring near the contact region with the glass substrate. In this framework, determination of the accurate distance from the surface to the plasma membrane is crucial to investigate the physical basis of cellular adhesion process. However, quantitative interpretation of TIRF pictures regarding the distance *z* between a labeled membrane and the substrate is not trivial. Indeed, the contrast of TIRF images depends on several parameters more and less well known (local concentration of dyes, angular emission pattern...). To get around this problem we propose to exploit a series of TIRF pictures recorded at different incident angles in evanescent regime. This technique called variable-angle TIRF microscopy (vaTIRF), allows mapping of the membrane-substrate separation distance with a nanometric axial resolution (10 nm) [1]. vaTIRF can be notably employed for real-time observations, which is important for any relevant investigations about cell adhesion and migration. We highlight that vaTIRF is useful not only to quantify the adhesion of living cells, but also to probe membrane-surface interactions. [1] R. Jaffiol, *Bio-physical Journal*, **111**, 1316-1327 (2016).

P-676

High-resolution imaging of living cells by atomic force microscopyA. Dulebo¹, I. Liashkovich², H. Schillers², A. Slade³, J. Shaw³¹Bruker Nano Surfaces Division, Germany; ²Institute of Physiology II, University of Münster, Münster, Germany; ³Bruker Nano Surfaces Inc, Santa Barbara, USA

Atomic force microscopy (AFM) provides the unique capability to obtain three-dimensional images of the morphology of individual living cells under physiological conditions without the need for labeling or staining. With the recent advances in high-speed AFM imaging, images could be obtained in a matter of seconds.

We have used a newly developed AFM probe, having a unique 17 micron tall tip and 65nm fixed end radius, together with the low piconewton imaging forces enabled by PeakForce Tapping mode to resolve individual microvilli structures on the surface of living cells for the first time by AFM. Changes in both the density and structure of microvilli are associated with various disease states. Our studies revealed a direct relationship of the observed structure of the microvilli with the interaction force of the AFM probe.

We have applied high speed AFM imaging to investigate the effect of a new compound called pitstop on Ea.hy926 endothelial cells' dynamics and motility. Pitstop has been shown to inhibit both clathrin-dependent and clathrin-independent endocytosis also having other plausible cellular targets. Here utilizing high spatial and temporal resolution we have been able to detect reversible inhibition of actin cytoskeleton dynamics and cell motility caused by pitstop.

P-678

Quantification of bright and dark-field scattering in cryo-scanning transmission electron tomographyA. J. Howe, D. Zbaida, S. G. Wolf, M. Elbaum
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Cryo-scanning transmission electron tomography (CSTET) can be used to obtain high-resolution three dimensional reconstructions of cellular ultrastructure in intact unstained vitrified cells. In CSTET, a focused electron beam is rastered across the sample and scattering signals can be simultaneously acquired on separate bright-field (BF) and dark-field (DF) detectors. Images acquired at a range of sample tilt-angles are reconstructed into a 3D volume. The incoherent nature of the signal collection allows us to relate pixel intensities from slices taken from BF and DF tomograms to the scattering from specific elements based on the defined angular collection ranges from the respective detectors. Quantification of the scattering can be demonstrated in model systems within cultured eukaryotic cells. The model systems used here are supramolecular assemblies of expressed Ferritin-YFP and the intracellular uptake of a modified fluorescent dye containing heavy metal atoms.

Posters**– 22. Imaging the cell –****P-679****Imaging of intracellular ATP level revealed pannexin1-mediated programmed ATP decrease in apoptosis**

H. Imamura, S. Sakamoto, T. Yoshida, A. Kakizuka
Kyoto University, Japan

Apoptosis is a form of programmed cell death and is considered as an energy-demanding process, requiring intracellular ATP for completing the cell death process. Because intracellular ATP of apoptotic cell is finally depleted, it seems likely that intracellular ATP level is tightly regulated during progression of apoptosis. However, dynamics and molecular mechanism of intracellular ATP decrease during apoptosis have not been well investigated. In this work, we achieved imaging of intracellular ATP level of single dying apoptotic cells by using a genetically encoded fluorescent ATP biosensor. It was observed that intracellular ATP levels started to decline after the activation of caspase-3. Strikingly, both pharmacological inhibition and RNAi knock-down of pannexin1 anion-channel, which is reported to release ATP and AMP after activation by caspase-3, strongly suppressed the decrease in intracellular ATP level during apoptosis. The decrease in intracellular ATP level was significantly accelerated by overexpression of wild-type pannexin1, but was abrogated by that of mutant pannexin1, in which the caspase-cleavage site was disrupted. Therefore, decrease in intracellular ATP level is most likely a programmed event in apoptotic cell death.

P-681**Measuring metabolic activities in single bacterial cells by Raman microspectroscopy**

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The bacterial metabolic activity differs among cells with an identical genome. The analysis of this individuality is essential for the understanding of their adaptation to environmental conditions, such as a persister cell phenomenon. We combined D₂O labeling with Raman microspectroscopy to measure metabolic activities in single bacterial cells. Cultured in a medium containing D₂O, active cells incorporate the deuterium and produce C-D bonds through metabolic processes, mainly the fatty acid biosynthesis pathways. The C-D bonds produce a detectable peak in the silent region of Raman spectra, allowing us to measure the gross metabolic activity. In order to confirm that the C-D peak reflects the metabolic activity, we measured dead bacterial cells treated with antibiotics. After incubation with D₂O, they showed lower C-D peak intensities than untreated cells. This means that the C-D peak can work as an indicator of the metabolic activity. By applying this strategy to cells cultured on the microscope, we also discovered that while dividing cells had high metabolic activities, most non-dividing cells were metabolically inactive. The result implies that this technique enables us to determine whether a cell is dead or alive, with no need for the observation of cell division.

P-680**Development of scanning microscope for simultaneous measurements of emission and excitation spectra**

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Tohoku University, Japan

We have developed a novel scanning microscope equipped with a function to measure emission, excitation spectra and corresponding images at the same time. White light continuum generated by a fs laser-pumped photonic-crystal fiber is dispersed by a prism and line focused on a sample. The excitation wavelength depends on the position along the line focus. The emissions excited by different wavelengths are collected at different heights of the polychromator entrance slit and detected as a 2D image on EMCCD, where vertical and horizontal axes correspond to the excitation and emission wavelengths, respectively. 3D imaging is achieved with a combination of a 2D piezo stage and a supersonic motor. We will demonstrate measurements of fluorescence images of fluorescent bead spin-coated on a glass surface, specimen of plant leaf, and green algae cells etc. with the developed system. We will report excitation spectra of photosynthetic proteins in these samples.

P-682**Porphyrin-based voltage-sensitive dyes for imaging and measuring membrane potential of cells**

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University of Oxford, UK

Current techniques for studying brain communication use microelectrodes and calcium indicators; however, these techniques have numerous shortcomings. Microelectrodes is a low throughput technique and do not provide high spatial resolution while, use of calcium indicators is an indirect technique thus resulting in poor temporal resolution. Fluorescence-based electrochromic voltage sensitive dyes (VSDs) give high background fluorescence and have issues of phototoxicity. Our group has previously shown that porphyrin-based VSDs which work on second harmonic generation (SHG), can be used to address the above-mentioned shortcomings. Here, we present a library of porphyrin-based dyes, which are expected to measure membrane potential of neurons through both fluorescence and SHG techniques. Some of the dyes show poor plasma membrane localization; however, they bind to cellular organelles giving SHG signals. Such dyes may be used to measure membrane potential of cellular organelles, which otherwise is not easily possible to measure. We also present a library of pyropheophorbide-based dyes, which can be useful to study cellular structures.

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P-683

Exploring the potential of Airyscan microscopy for live cells imagingK. Korobchevskaya¹, B. C. Lagerholm², M. Fritzsche^{1,2}¹Kennedy Institute of Rheumatology, University of Oxford, UK; ²Weatherall Institute of Molecular Medicine, University of Oxford, UK

Biological research increasingly demands the use of non-invasive and ultra-sensitive imaging techniques. The Airyscan approach was recently developed to cover the gap between conventional confocal and super-resolution microscopy techniques [1]. It exploits the pixel reassignment principle in order to enhance both resolution and signal to noise ratio (SNR) without increasing the excitation power and acquisition time, which is especially useful for live cell imaging. The main feature of the Airyscan system is a 32 channel GaAsP detector, which allows simultaneous acquisition of multiple data points for subsequent image reconstruction. Here we present a detailed study of Airyscan (ZEISS LSM 880) performance in comparison with conventional confocal mode by imaging reference samples with different acquisition and processing parameters. We found that for bright samples the Airyscan method shows resolution comparable with confocal imaging at 0.2AU, but with significantly improved SNR; which indicates a great potential for live cell dynamical studies. However, great attention should be paid to the levels of applied filtering, as high values might result in significant data loss and image distortion.

1. Weisshart, K. The Basic Principle of Airyscanning. *Zeiss Microsc.* 22 (2014)

P-685

Optical scattering and microscopic imaging of cellular exo- and endocytosisD. Marques¹, A. Miranda³, A. G. Silva¹, P. Munro², P. de Beule³¹Universidade Nova de Lisboa, Portugal; ²University College London, UK; ³International Iberian Nanotechnology Laboratory, Portugal

We demonstrate that white light microscopic observation of cellular exo- or endocytosis is affected by lipid induced optical anisotropy through the application of an exact electromagnetic model for image formation. First, we present a solution to Maxwell's equations for core-shell particle scattering near an isotropic substrate covered with an anisotropic thin film, based on an extension of the Bobbert-Vlieger solution for particle scattering near a substrate. We apply this solution to the calculation of light scattering of a lipid vesicle near a lipid bilayer whereby the lipids are represented by a biaxial optical model representative for lipid induced optical anisotropy. We use ellipsometry concepts to demonstrate that lipid induced anisotropy effectively alters the far-field scattering. Furthermore, we have integrated this Bobbert-Vlieger scattering solution into a rigorous model of Differential Interference Contrast (DIC) image formation which allows for characterising DIC, through simulation, as a tool for imaging of exo- or endocytosis events. We compare theoretical predictions with experimental high numerical aperture DIC imaging of dielectric oxide nanoparticles with organic shell.

P-684

Impact of fixation on the cell-substrate distance studied with Surface Plasmon Resonance Microscopy

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The characterization of the cell-substrate interface is of particular importance in the fields of bioelectronics, biomechanics, cell engineering and biomedical research. Common characterization methods such as focussed ion beam cutting (FIB) combined with electron microscopy (SEM or TEM) require fixation or critical point drying which might affect the membrane topology and thus affect the cell-substrate distance. Therefore, we introduced a lens-type scanning surface plasmon resonance microscope (SPRM). The excitation of localized surface plasmons allows us to achieve a lateral resolution beyond the refraction limit. With spectroscopic data analysis we are able to determine the cell-substrate distance in vitro and without any labelling with a resolution in the nm range in z-direction. We studied the effect of chemical fixation by measuring the same cells with the SPRM in vitro and after a fixation with glutaraldehyde. We observed a significant decrease of the cell-substrate distance after fixation and could confirm these measurements with SEM images of FIB cuts of the fixed cells. These results may question the reliability of fixation based methods for the characterization of the cell-substrate distance at the interface.

P-686

Insights of electroporated cells as revealed by digital holographic microscopyM. G. Moisescu¹, M. Mihailescu², V. L. Calin¹, A. V. Baluta², E. Scarlat², E. Kovacs¹, T. Savopol¹¹Carol Davila University of Medicine and Pharmacy of Bucharest, Faculty of Medicine, Biophysics and Cellular Biotechnology Dept., Romania; ²Politehnica University of Bucharest, Faculty of Applied Sciences, Physics Dept., Romania

Electroporation is a platform technology for various biomedical applications. Cellular and molecular changes due to membrane permeabilization and consecutive recovery require however a better understanding. Using an *off-axis* digital holographic microscope, optical and shape-related characteristics of B16F10 cells were investigated after trains of bipolar rectangular pulses (1kV/cm, 100µs, 1kHz). The refractive index *RI* and cell height were calculated in a specifically defined area using the *decoupling procedure* and the global cell parameters (projected area *A*, averaged optical phase shift *OPS* and dry mass *DM*) were monitored for 10min. At 2s after pulse delivery, the cell height increased by 33%, *RI* dropped by 1.2%, the latter recovering and reaching the controls value after about 4min. The biphasic evolution of cellular *A* and *OPS*, while *DM* remained unchanged, have been discussed by solutes dynamics through the electroporated cell membrane.

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– 22. Imaging the cell –

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Holotomography (HT) techniques for non-invasive label-free 3D imaging of live cells and tissues

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Holotomography (HT) uses a laser interferometry to measure 3-D refractive index (RI) distribution. HT serves as a powerful tool for imaging small transparent objects, such as biological cells and tissues. HT is an optical analogous to X-ray computed tomography (CT); HT measured multiple 2-D holograms of a sample with various illumination angles, from which a 3-D RI distribution of the sample is reconstructed by inversely solving the wave equation.

Unlike conventional fluorescence-based imaging techniques, HT provides label-free 3-D imaging capability. Without any fixation or labeling, 3-D images of live cells can be obtained with high spatial resolution (down to 110 nm). Furthermore, HT provide quantitative imaging capability: RI maps of a cell are precisely and quantitatively measured, from which various cellular analysis can be followed.

In this talk, we will present the recently developed 3-D holotomography setup using a dynamic mirror device. In particular, we will discuss the principle of HT techniques and the previous application in the field of hematology, cell biology, neuroscience, and infectious diseases [1–4]. The outcome demonstrates outstanding visualization of 3D refractive index maps of live cells, which will be potentially used in various applications in biology and medicine. We will also discuss about the commercialization of the technique.

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P-689

The Research on the Autophagy and Its Markers in Hippocampal Neurons of Rats induced by Microwave Radiation

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The brain is one of the target organs sensitive to microwave radiation. Autophagy can remove intracellular aging or damaged organelles and proteins, and its role in synaptic plasticity is gradually taken seriously. The aims was to observe the dynamic changes of autophagy and the expression of its markers in hippocampal neurons of rats after microwave radiation. In the study, 104 male Wistar rats was exposed to 30mW/cm² microwave. The change of learning and memory ability was detected by Morris water, LTP, hippocampus tissue structure and ultrastructure were detected respectively, and the changes of LC3-II/I, Beclin1, LAMP1, Atg5, Atg7 and Atg9 were detected by immunoblotting in rat hippocampus within 3m after radiation. Results showed that 30mW/cm² microwave radiation caused synaptic plasticity injury in rats, manifested as spatial learning and memory ability decline, LTP induction disorder and synaptic ultrastructural damage; Autophagy in hippocampal neurons was activated, autophagosomes and autolysosomes were increased and the expression of related markers elevated after 30mW/cm² microwave radiation.

Key words: microwave radiation; hippocampus; synaptic plasticity; autophagy.

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P-688

Death or alive? Correlating the cell wall structure of sacculi and living bacteria using AFML. Pasquina Lemonche¹, S. Kumar¹, R. D. Turner², J. M. Burns¹, N. Mullin¹, R. K. Tank¹, D. S. Owen¹, S. J. Foster², J. K. Hobbs¹

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This project is focused on *Staphylococcus aureus* (*S. aureus*), a pathogen capable of provoking a wide range of human diseases. *S. aureus* has evolved into bacteria resistant to antibiotics (e.g. *penicillin*) and is crucial solve fundamental questions of this specimen. To study this bacteria Atomic Force Microscopy (AFM) has been used. AFM can produce high resolution images at nanometer scale of biological samples without damaging or altering their surface. Allowing us mapping the structure of peptidoglycan (cell wall of Bacteria).

Direct imaging of the cell wall in previous studies were used to build models of peptidoglycan molecular structure. These studies were performed using living cells and purified peptidoglycan material from death cells, called sacculi. A significant advantage of imaging sacculi is the direct visualization of inner cell wall (not possible in living cells). However, all the previous studies of sacculi have been done in dry environment. The drying process can induce changes in peptidoglycan structure. Therefore, in this project a methodology has been developed to image sacculi in liquid environment and correlate the results with living cells. This will improve our understanding of the native structure of this biopolymer.

P-690

Nucleoid reorganization of H-NS in response to environmental stressN. Rafiei^{1,3}, W. Navarre², J. N. Milstein^{1,3}

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In this study, we investigated how exposure of *E. coli* to environmental stress influences the spatial distribution of the heat-stable nucleoid structuring (H-NS) protein along the chromosome. H-NS is an abundant histone-like DNA-binding protein in Gram-negative bacteria. It preferentially binds to AT-rich domains of DNA and affects both global gene regulation and DNA packaging¹. To understand how H-NS is reorganized under different conditions, we applied osmotic stress and cold-shock (21°C) on bacterial cells at different phases of growth: log phase, early and late stationary phases. To visualize H-NS, the *hns* gene in the chromosome was fused to the fluorescent protein mEos3.2, which enabled us to perform photo-activated localization microscopy (PALM). We observed that the intracellular organization of H-NS started to alter under all conditions in the early stationary phase, however, the response was most profound under osmotic stress. The question then arose as to whether this response to osmotic stress is due to an architectural change of the chromosome or a corresponding redistribution of H-NS. To answer this question, we then explored the organization of HU in the same bacteria strains in which the *hu* gene in the chromosome was fused to mEos3.2. HU is another histone-like DNA-binding protein which binds non-specifically to DNA². Therefore, visualizing the spatial distribution of HU could provide insight into the DNA architecture.

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Posters

– 22. Imaging the cell –

P-691

Unlabelled super-resolution imaging using polarisation-contrast super-oscillatory microscopyE. T. Rogers¹, S. Quraishi², T. A. Newman², J. E. Chad³, N. I. Zheludev^{1,4}, **P. J. Smith³**¹Optoelectronics Research Centre, University of Southampton, UK; ²Clinical and Experimental Sciences, University of Southampton, UK; ³Biological Sciences, University of Southampton, UK; ⁴Centre for Disruptive Photonic Technologies, Nanyang Technological University, Singapore

Super-resolution microscopy is an important new tool for the biosciences, but current techniques require addition of fluorescent probes. We have developed a new technique for optical super-resolution imaging of unlabelled living cells.

Optical super-oscillations allow us to create an arbitrarily small hotspot using precisely-engineered interference of light. Super-oscillatory hotspots are, however, surrounded by sidebands that contain a fraction of the optical power – trading efficiency for resolution. We replace the conventional focusing lens in a confocal microscope with a super-oscillatory lens and use the pinhole to reject the light scattered from the sidebands, giving us an image with resolution determined by the size of the super-oscillatory hotspot.

To image unlabelled cells, we combine this with an advanced form of polarisation-contrast imaging. We capture four super-resolved images of the sample with different incident polarisations, from which we calculate the anisotropy magnitude and orientation. This highlights those parts of a cell with significant molecular structuring, such as actin filaments, microtubules, and even protein enriched lipid bilayers such as vesicles and cell membranes.

P-693

Three-dimensional protein dynamics in the cell nucleusA. P. Singh¹, R. Galland^{2,3}, M. L. Finch-Edmondson¹, G. Grencl¹, J.-B. Sibarita^{2,3}, V. Studer^{2,3}, V. Viasnoff^{1,4}, **T. E. Saunders¹**¹Mechanobiology Institute, National University of Singapore, Singapore; ²Institut Interdisciplinaire de Neurosciences, University of Bordeaux, France; ³Centre National de la Recherche Scientifique UMR 5297, University of Bordeaux, France; ⁴Centre National de la Recherche Scientifique UMI 3639, Singapore

The three-dimensional (3D) architecture of the cell nucleus plays an important role in protein dynamics and in regulating gene expression. However, protein dynamics within the 3D nucleus are poorly understood. Here, we present, to our knowledge, a novel combination of 1) single-objective based light-sheet microscopy, 2) photoconvertible proteins, and 3) fluorescence correlation microscopy, to quantitatively measure 3D protein dynamics in the nucleus. We are able to acquire >3400 autocorrelation functions at multiple spatial positions within a nucleus, without significant photobleaching, allowing us to make reliable estimates of diffusion dynamics. Using this tool, we demonstrate spatial heterogeneity in Polymerase II dynamics in live U2OS cells. Further, we provide detailed measurements of human-Yes-associated protein (YAP) diffusion dynamics in a human gastric cancer epithelial cell line. We are using this approach to explore the dynamics of the Hippo-YAP signaling pathway under different genetic and mechanical conditions.

P-692

Intracellular local thermogenesis initiates stress granule formation**B. Shi¹**, K. Okabe^{1,2}, T. Funatsu¹¹Graduate School of Pharmaceutical Sciences, the University of Tokyo, Japan; ²JST, PRESTO, Japan

Under stress, eukaryotic cells form stress granule (SG), which is responsible for translation regulation. Recent studies suggest that SG assembly involves phase separation and self-aggregation of its components. The initiation mechanism, however, still remains uncertain. In this study, we supposed that local temperature change in the cytoplasm influences the dynamic behavior of mRNA and drives an interaction of SG components, and therefore, might be a critical trigger of SG formation. Thus, we investigated the contribution of intracellular temperature change on SG formation. By measuring the intracellular temperature in stressed cells, we confirmed that temperature increases during SG formation. To further investigate, we established a method of manipulating intracellular temperature, and our observations revealed that SG formation can be induced by local temperature increase alone, indicating that the intracellular local temperature change serves as a trigger for the initiation of SG formation. By clarifying the relationship of eIF2 α phosphorylation, the well known critical step for initiating SG assembly, we suggested that intracellular thermogenesis and eIF2 α phosphorylation synergistically enhance SG formation.

P-694

How does fluorophore saturation influence intensity-based FRET calculations?**T. Szendi-Szatmari¹**, A. Szabo², J. Szollosi^{1,2}, P. Nagy¹¹Department of Biophysics and Cell Biology, University of Debrecen, Hungary; ²MTA-DE Cell Biology and Signaling Research Group, University of Debrecen, Hungary

We examined the effect of fluorophore saturation on intensity-based FRET calculations. In confocal microscopy the intensity of excitation is sufficient to result in singlet- and triplet-state saturation. This phenomenon undermines some of the assumptions made in the evaluation of intensity-based FRET measurements. Cells were labeled with fluorescent antibodies against two different epitopes of ErbB2 followed by calculating the FRET efficiency at different laser intensities in microscopy. The results show that the FRET values decrease with the increase of the laser intensity. We attribute this result to fluorophore saturation. We compared the results of flow cytometric and microscopic measurements. In contrast to our expectations the FRET values determined by flow cytometry, where fluorophore saturation does not take place, are lower than in microscopy. We used an oxygen scavenging-system and triplet state quenchers in order to modify the fraction of dyes in the triplet state. Our preliminary results show that O₂ scavengers decrease the dependence of the calculated FRET efficiency on the excitation intensity. Although intensity-based FRET calculations are widely used, the effect of fluorophore saturation is often overlooked which may lead to misleading FRET values.

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– 22. Imaging the cell –

P-695

Intracellular drug imaging by stimulated Raman scattering microscopyW. J. Tipping^{1,2}, M. Lee², A. Serrels², V. G. Brunton², A. N. Hulme¹¹School of Chemistry, The University of Edinburgh, UK; ²Institute of Genetics and Molecular Medicine, The University of Edinburgh, UK

Rapid advances in the field of Raman imaging, particularly stimulated Raman scattering (SRS) microscopy are opening up many new avenues for imaging and quantification of small molecules in living systems. These advances mean that for the first time, images of small molecules within cells might be acquired without the use of “bulky” fluorescent labels, (which may be as big as the small molecule under observation) or the use of nanoparticle sensors (which might perturb cellular biology).

The detection of compounds with low intracellular concentrations remains challenging. In these instances, a Raman labelling approach may facilitate detection. However, a modular workflow for the rational design and evaluation of potential Raman labels is yet to be established, which would allow SRS microscopy to be fully exploited in the drug development process. Here, we present highly Raman active functional groups for drug labelling and imaging by SRS microscopy. This approach has enabled the rate of drug uptake to be assessed in real-time. Furthermore, combining multi-colour SRS microscopy and dual-modality imaging allows drug accumulation to be mapped across intracellular structures. These studies clearly demonstrate the potential for SRS microscopy in the drug development process.

P-697

Probing the mechanism of bacterial flagella assembly by real-time fluorescent imagingX.-Y. Zhuang¹, Z. Zhao², M. Homma³, S. Kojima³, F. Bai², C.-J. Lo¹¹Department of Physics, National Central University, Taiwan; ²Biodynamic Optical Imaging Center, Peking University, China; ³Division of Biological Science, NAGOYA UNIVERSITY, JapanE-mail: cjlo@phy.ncu.edu.tw

Bacterial flagella are self-assembled external helical filaments for swimming. During the assembly process, flagellins are transported unfolded through the central channel in the flagellum to the growing tip. We develop *in vivo* single-cell fluorescent imaging technique to monitor in real time the *Vibrio alginolyticus* polar flagella growth. The flagellar growth rate is found to be highly length-dependent. We characterized the key factors controlling the flagellar growth rate. We modeled the flagella growth as a one-dimensional injection-diffusion system. When the flagellum is short, its growth rate is determined by the loading speed at the base. Only when the flagellum grows longer does diffusion of flagellin become the rate-limiting step, dramatically reducing the growth rate. Our first real-time visualization of flagellar growth and biophysical model shed new light on the dynamic building process of this complex extracellular structure.

Reference: Length-dependent flagellar growth rate of *Vibrio alginolyticus* revealed by real time fluorescent imaging. *eLife*. e22140 (2017)

P-696

Biophysical properties of plastoglobules isolated from Arabidopsis mutantsJ. Wójtowicz¹, J. Grzyb², K. B. Gieczewska¹¹Faculty of Biology, University of Warsaw, Poland; ²Institute of Physics PAS, Warsaw, Poland

Plastoglobules are lipoprotein particles inside chloroplasts. Surrounded by a monolayer membrane they have unique spatial relationship to thylakoid membranes. They number and size may varied during changes induced by chilling, excess of light, senescence or oxidative stress. Plastoglobules actively participate in thylakoid function and they rearrangement. It is quite interesting do biophysical properties of plastoglobules are similar to the thylakoid ones, in different plant species. We used mostly the Arabidopsis mutants in various genes, some involved in lipid synthesis and composition, some more susceptible for chilling. To investigate the topography and physical properties of these structures contact and non-contact mode of AFM microscope was used. We also try to describe elasticity of plastoglobules as well as type of interactions between protein and lipids within plastoglobules' monolayer.

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Posters

– 23. Biomimetic structures and systems –

P-698 (O-137)

Protein assembly, from small molecule to polymer mediators

P. B. Crowley
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Protein assembly triggered by supramolecular building blocks offers fascinating routes to hybrid bio-inspired materials. We are using calixarenes as “molecular glues” to mediate protein assembly. A combined methodology of NMR spectroscopy, X-ray crystallography and SEC-MALS has provided convincing evidence that sulfonato-calix[n]arenes are versatile ligands for protein surface recognition and assembly.[1–3] New examples of calixarene-mediated assembly will be presented.

Recently, we showed that PEGylation can result in highly porous protein assemblies.[4] Current efforts are focused on protein interactions with PEGylated-calixarenes, which also yield porous assemblies as revealed by X-ray crystallography.

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P-700 (O-136)

Characterization of matrix vesicles biomimetic systems: interaction with collagen fibers during biomineralization

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Osteoblasts control the deposition of the extracellular matrix and the release of matrix vesicles (MVs), which serve as the initial sites for hydroxyapatite formation. Annexin V (AnxA5) is a phospholipid-dependent Ca^{2+} -binding protein, which acts as Ca^{2+} -channel in the MVs' membrane. Tissue-nonspecific alkaline phosphatase (TNAP) is attached to the MVs' outer membrane and acts as a pyrophosphatase and ATPase producing P_i and regulating the mineralization. We describe the preparation of DPPC and DPPC:DPPS (9:1) proteoliposomes harboring AnxA5, TNAP or AnxA5+TNAP, and their use as MVs mimetic systems. Enzymatic activity and Ca^{2+} uptake validated the functional incorporation of both proteins in mimetic systems. AnxA5-proteoliposomes bound type II collagen with the highest affinity when compared with collagen I and III. The presence of DPPS significantly enhanced the binding up to 74%. TNAP-proteoliposomes for both lipid compositions poorly bound the collagen matrix (< 20%). Proteoliposomes harboring both proteins showed 30% of binding. The interaction between AnxA5 and type II was Ca^{2+} independent. These findings suggest that AnxA5 has a double role, create a Ca^{2+} -rich environment inside MVs and anchor MVs to collagen at calcification sites. CNPq, CAPES, FAPESP.

P-699 (O-138)

Super-resolution DNA-origami barcodes: a labeling system for spatially resolved deep-sequencing

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It has been demonstrated with the emergence of single-cell sequencing techniques that adjacent cells in tissues can show a remarkable diversity in terms of gene activity, which was previously impossible to detect with bulk experiments. One of the current main efforts of the single cell transcriptomics field is to link spatial information to single cell transcription data. In the presented work, we describe the use of DNA-origami for the development of a multiplexable tagging-system, detectable by next-generation sequencing (NGS) as well as super-resolution microscopy, with the potential to uniquely label a great number of cells in tissue-samples and permitting the coupling of the RNA-seq data of individual cells to positional information through the use of microscopy images of barcoded samples. The described tagging-system consist of a library of geometrically encoded, super-resolution DNA-origami barcodes, constructed in a combinatorial fashion, that can be read out using super-resolution microscopy and NGS techniques as well, through the encoding of the barcodes' optical code into the sequence of their scaffold DNA molecules. The labeling-system is aimed to be adapted as a preceding labeling and imaging step to the already established single-cell RNA-seq protocols.

P-701

Lipodisq and native mass spectrometry: a new tool for studying membrane proteins in native environment

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Biophysical characterisation of membrane receptors in their native environment is essential for drug development, as lipid removal may affect conformational freedom and ligand binding. Membrane proteins are commonly solubilised with detergent for structural and functional studies, although the resulting micelles may be prone to aggregation, occlusion of the binding site and polydisperse. Recently a hydrolysed copolymer of styrene and maleic acid (SMA) has been shown to be highly effective in extracting membrane proteins from native membranes without the use of detergent and the resulting coin-shaped nanoparticles, called Lipodisqs (or SMALPs) are suitable for use in a wide range of biophysical methodologies. Here we have been able to purify and characterize a range of membrane proteins from their native environment including GPCRs, transporters and channels using size-exclusion and affinity chromatography, circular dichroism and dynamic light scattering. Microscale thermophoresis and radio-ligand binding have been used to assess the activity of the different receptors. These nanoparticles have further allowed us to characterize oligomeric state and to identify native ligands by native ESI mass spectrometry.

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– 23. Biomimetic structures and systems –

P-702

Cholera Toxin binding to GM1 in raft compositions using Microcavity Suspended lipid bilayers
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Cholera toxin, gains entry to the mammalian cell through specific associative interactions with membrane components. Its B-subunit (CTxB) comprises five identical peptides that form a pentameric ring capable of binding up to 5 GM1 gangliosides on intestinal epithelial cells. Generally, GM1 is found in lipid rafts, liquid ordered domains rich in sphingolipids (SM) and cholesterol. This study presents an innovative micro cavity array device used to address specific CTxB-GM1 and CTxB-raft interactions. Asymmetric lipid bilayers containing GM1 in outer leaflet and symmetric lipid bilayers mimicking lipid rafts composition were built above aqueous filled micro cavities using Langmuir-Blodgett method followed by liposome disruption. The CTxB labelled Alexa-555 is introduced in the platform and the fluorescent probe is analysed by Fluorescence Lifetime Correlation Spectroscopy. Overall, we find lateral diffusion values for GM1-CTxB binding in a DOPC lipid bilayer is $4.94 \pm 0.90 \mu\text{m}^2 \text{s}^{-1}$. Surprisingly, the CTxB lateral diffusion without GM1 in raft composition and DOPC/SM is $4.55 \pm 1.10 \mu\text{m}^2 \text{s}^{-1}$ and $4.64 \pm 1.95 \mu\text{m}^2 \text{s}^{-1}$, respectively. These observations suggest that the platform is suitable to study the CTxB-GM1 binding and its potential use for biomedical applications.

P-704

Topographic analysis by AFM of proteoliposomes matrix vesicle mimetics harboring TNAP and AnxA5M. Bolean¹, I. A. Borin¹, A. M. S. Simão¹, M. Bottini^{2,3}, J. L. Millán³, P. Ciancaglini¹¹USP, Ribeirão Preto, Brazil; ²University of Rome Tor Vergata, Italy; ³SBP Medical Discovery Institute, USA

Atomic force microscopy (AFM) is one of the most commonly used scanning probe microscopy techniques for nanoscale imaging. The present study extends the capabilities of AFM to the characterization of proteoliposomes, a special class of liposomes mimicking matrix vesicles (MVs) involved in the biomineralization process. We describe the preparation of DPPC:DPPS (9:1) proteoliposomes harboring tissue-nonspecific alkaline phosphatase (TNAP) and/or annexin V (AnxA5), both characteristic proteins of osteoblast-derived MVs. AFM images acquired in dynamic mode revealed the presence of microdomains with distinct viscoelasticity, thus suggesting that the presence of the proteins induced local changes in membrane fluidity, compatible with the generation of microdomains prominent in TNAP-proteoliposomes, but barely detectable in AnxA5-proteoliposomes. A more complex microdomain structure was observed for the mixed TNAP+AnxA5-proteoliposomes, resulting in a lower affinity for type II collagen fibers for the mixed proteoliposomes, compared to exclusive AnxA5-proteoliposomes. The present study achieved an improved the topographic analysis of lipid vesicles by direct visualization of structural changes, resulting from proteins incorporation. CNPq, CAPES and FAPESP.

P-703

Delivery of membrane proteins into membrane mimicking systems by charge-mediated fusion

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During oxidative phosphorylation, membrane embedded complexes I to V functionally interact to form ATP from cellular reducing equivalents. Normally, these enzymes are investigated individually and therefore, their functional interplay is not yet understood in detail. To get further insights on interactions of respiratory chain enzymes, they have to be co-reconstituted into a well-defined membrane mimicking system. Recently, we found that if two liposome populations either containing negatively or positively charged lipids were mixed, they rapidly undergo fusion yielding a liposome containing both membrane proteins. Using this strategy, it was possible to co-reconstitute different terminal oxidases and the *E. coli* ATP synthase, imitating the last step of oxidative phosphorylation. Next, we applied this technique to deliver ATP synthase, reconstituted into positively charged liposomes, into native inverted membrane vesicles of *E. coli* (which are negatively charged) that lack this enzyme. Upon fusion, respiratory driven ATP synthesis was restored. However, positively-charged lipids are not found in natural membranes what might influence membrane proteins. To overcome this obstacle we currently test ionisable lipids, which are only positively charged below a certain pH threshold.

P-705

Investigating sequence, structure and function relationships in antimicrobial peptoids

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Peptoids (or N-substituted glycines) are peptidomimetic molecules which are being increasingly investigated for the pharmaceutical properties as novel anti-infectives, biomimetic materials and drug delivery vehicles. As for the peptides they mimic, peptoids have been shown to adopt defined 3D structures although these structures are not fully characterised.

Here we discuss the use of a range of biophysical characterisation techniques to expand understanding of how peptide sequence encodes folding and stability, which is then implicated in drug efficacy. Specifically we demonstrate that folding has an important impact on the apparent hydrophobicity of the molecules and their ability to penetrate model membranes.

A library of peptoids has been made and evaluated in which different patterns of hydrophobic and hydrophilic residues are utilised. The impact of these sequence modifications on the structure and biophysical properties of the molecules is evaluated.

Posters

– 23. Biomimetic structures and systems –

P-706

Photo-induced oxidation of bio-mimetic membranes : Giant pore openings and membrane defectsA. Bour¹, S. Kruglik¹, N. Puff², S. Bonneau¹¹Laboratoire Jean Perrin, UMR8237 UPMC-CNRS, France;²Laboratoire MSC, UMR7057 UPD-CNRS, France

Bio-membrane oxidation is an important phenomenon involved in aging, degenerative diseases, cell apoptosis and mitochondrial functioning. Here we study lipid oxidation effects depending on the number of insaturations along the aliphatic chains and the lipid headgroups.

Our study consists in following photo-induced oxidation of model membranes of different compositions by two experimental set-ups. The first setup enables us to characterize the ability of vesicles to resist to oxidation. Changes in phase contrast of giant vesicles allow to determine the membrane permeation caused by oxidation. Typically when oxidation starts, contrast fluctuates for a time t_0 called starting permeation time, then decreases exponentially with a characteristic time used to define a photo-induced permeability. The second setup gives us access to the chemical scenario of oxidation. Optical tweezers are coupled to a Raman spectroscope.

Surprisingly, our results show that the number of insaturations does not influence t_0 . Second, a small amount of cardiolipin postpones oxidation effects. Moreover, we propose two mechanisms to explain the different permeabilisation behaviours (corresponding to different membrane compositions) : a "membrane-defect" mechanism, and a "giant pore openings" mechanism.

P-708

Role of membrane sphingolipids in the interaction with amyloid beta-peptideR. Carrotta¹, F. Librizzi¹, M. R. Mangione¹, S. Vilasi¹, C. Cossu², O. Moran²¹CNR - Istituto di Biofisica - Via Ugo La Malfa 153, 90146 Palermo, Italy; ²CNR - Istituto di Biofisica - Via de Marini 6, 16149 Genova, Italy

The early impairments appearing in Alzheimer's disease are related to neuronal membrane damage. Interaction of the amyloid A β -peptide (A β) with the phospholipid bilayer cause the onset of pathological mechanisms. Both, aberrant A β species and membrane components play a role in promoting aggregation, deposition and signal dysfunction. Ganglioside GM1, present with cholesterol and sphingomyelin in lipid rafts, seems to be able to initiate A β aggregation on membrane. Interaction of liposomes, as membrane models, with A β was studied by isothermal titration calorimetry and SAXS, to couple thermodynamic and structural information. Liposomes made with PC:PS:Chol (8:1:1) and 5% GM1 (100nm LUV) interact with A β at pH 7.4 by changing the double layer structure, thus indicating the recruitment of A β toward the membrane. No interaction was measured when GM1 was not embedded in the liposomes, indicating an electrostatic driven selective interaction between GM1 and A β . The interaction between liposomes and A β depends on the GM1 percentage. To mimic a more realistic lipid composition of the lipid rafts, we also analysed interaction of liposomes enriched with sphingomyelin or ceramide, with A β . SAXS analysis evidence structural differences among liposomes with different composition.

P-707

Monitoring conformational states and kinetics of DNA origami objects by small-angle X-Ray scatteringL. K. Bruetzel¹, P. U. Walker¹, T. Gerling², H. Dietz², J. Lipfert¹¹LMU Munich, Nanosystems Initiative Munich, Center for Nanoscience, Germany; ²TUM Munich, Walter Schottky Institute, Germany

Molecular self-assembly of DNA, so-called DNA origami, is a promising approach for creating versatile structures at the 1-100 nm scale and in the MDa size range that has allowed to explore novel applications in many directions, such as guided material assembly, nanorobotics, disease diagnosis, and drug delivery.

While an important initial focus in the design of self-assembled DNA structures was to create static objects of precisely defined two- and three-dimensional shapes, recent emphasis has shifted to constructing dynamic structures that can change shape in response to environmental conditions. Such functional and dynamic origami structures require techniques that can probe and resolve their conformational changes and three-dimensional structures, ideally in solution, free of potential biases from surface attachment or labeling. Here, we demonstrate that small-angle X-ray scattering (SAXS) can quantitatively resolve the structures of complex 3D homo- and heteromultimeric DNA origami devices under varying solution conditions. Moreover, we were able to monitor and characterize the conformational kinetics of these objects on different timescales.

P-709

Lipid-based adjustable-gradient nanopores for single molecule detection and manipulationK. Chekashkina^{1,2}, P. Kuzmin², S. Romanov¹, T. Galimzyanov², V. Frolov^{3,4}, P. Bashkirov^{1,2}¹Federal Research and Clinical Center of Physical-Chemical Medicine, Russian Federation; ²Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Federation; ³Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, UK; ⁴IKERBASQUE, Basque Foundation for Science, Spain

Thanks to the latest advancements in nanotechnology and molecular engineering, development of synthetic systems that mimic biological processes and structures has now become possible at the nanoscopic level (on a nanoscale). Such an assimilation of ideas and functional elements from living nature looks especially promising for development of various nanofluidic systems designed for achieving certain properties and behaviors of fluids confined by nanometer-scale structures. Thus, in order to improve transport properties of solid-state nanopores their walls could be passivated with a lipid bilayer film, which behaves as a two-dimensional liquid and is capable of supporting transport phenomena. In the present work, we demonstrated that electrostatic potential gradient forced charged membrane components to redistribute along a lumen of membrane cylindrical nanotube (NT) pulled from planar membrane. We utilized this property of NT membrane to produce adjustable gradient nanopores for single molecule detection and manipulation. This work was supported by RSF grant 14-14-01001.

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– 23. Biomimetic structures and systems –

P-710

Capsaicin and local anesthetics interaction with model membranes

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Local anesthetics (LA) are amphiphilic molecules often used in medical practice. Capsaicin is a lipophilic molecule found in peppers that bind to the vanilloid receptor TRPV1 (painful stimuli). Recently, it was reported that the application of LA followed by capsaicin leads to an increase in the sensory blockade in rats due to facilitated LA entrance into nociceptive fibers (Gerner, 2008). Here we tested the effect of LA and capsaicin on model membranes composed of POPC, SM and CHOL, 2:1:2mol%. The incorporation was followed by phase-contrast and fluorescence microscopy of giant unilamellar vesicles (GUV). Incorporation of capsaicin in the bilayer caused an increase in the surface area. GUV response somehow differ among the tested LA (mepivacaine, lidocaine, and bupivacaine) with high doses of LA immediately causing formation of micrometer-sized domains. Moreover, incorporation of capsaicin and LA led to accentuated phase separation, detaching of the fluid (*L_d*) lipid fraction from the vesicle. These results suggest that the prolonged analgesic effect achieved after capsaicin+LA administration may also be related to rearrangements in the lateral phase distribution of lipids. Financial support: FAPESP15/11804-9, 14/14457-5, 15/09948-2, CAPES.

P-712

Fast collisional lipid transfer among polymer-bounded nanodiscs

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Copolymers of styrene/maleic acid (SMA)¹ or diisobutylene/maleic acid (DIBMA)² can solubilise membrane proteins and surrounding lipids to assemble into polymer-bounded nanodiscs. Although the latter preserve a lipid-bilayer core, they are much more dynamic than other membrane mimics. By using time-resolved FRET and SANS, we determined the kinetics and unravelled the mechanisms of lipid transfer among these nanodiscs.³ Unlike other bilayer systems, polymer-bounded nanodiscs exchange lipids not only by monomer diffusion but also by fast collisional transfer. Under typical conditions, lipid exchange occurs on the timescale of seconds in the case of polymer-bounded nanodiscs but takes minutes to days in protein-bounded nanodiscs or vesicles. The independence of the rate constants of probe hydrophobicity and the low activation enthalpy of lipid exchange³ point to the transient formation of a “hydrocarbon continuum” during collisional transfer. Thus, polymer-bounded nanodiscs represent highly dynamic equilibrium rather than kinetically trapped assemblies, which is relevant for studying protein/lipid interactions.

¹Knowles et al. *J. Am. Chem. Soc.* **2009**, *131*, 7484²Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919³Cuevas Arenas et al. *Sci. Rep.* **2017**, in press

P-711

Measuring dielectric constants of lipid bilayers: monitoring scatter effects in pyrene fluorescence

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Pyrene (Py) fluorescence is expedient to study biophysical phenomena in lipid bilayer due to high quantum yield, long lifetime and precise solvatochromic effects [1]. The physical-chemical properties of Py settle its location in the ordered section of the methylenic palisade stating the values of dielectric constants of bilayers, averaged transversally in space (the longest axis of Py, 9.2 Å) and laterally in time (due to lateral diffusion of Py during its lifetime, 150 ns) [2]. To do so, the fluorescence spectra must be corrected for the turbidity from liposome suspensions that fatally affects the measurements of Py fluorescence. Analogous procedure is adopted to settle a reference plot for Py fluorescence in isotropic alcohols of known dielectric constants at 20 °C.

The bilayer polarity of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and its binary mixtures with cholesterol were monitored using the Py Ham Effect (I_1/I_3). Pure POPC exhibits higher dielectric constant than the mixtures at high cholesterol proportions, pointing to features observed in the available thermal phase diagrams.

References:

[1] D. Arrais, J. Martins (2007) *Biochim. Biophys. Acta* **1768**:2914-2922.[2] L.M.S. Louira, A.M.T. Martins do Canto, J. Martins (2013) *Biochim. Biophys. Acta* **1828**:1094-1101.

P-713

Lateral heterogeneity of the membrane liposomes in the present of plant polyphenols

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We studied the effects of plant polyphenols (phloretin, phlorizin, quercetin, and myricetin) on the domain structure of liposomes mimicking the membranes of fungi, mammalian and plant cells. Using the fluorescence confocal microscopy of giant unilamellar vesicles composed of ternary mixtures of low-melting phospholipid (DOPC, POPC, or DPOPC), high-melting lipid (sphingomyelin, DPPC, or tetramyristoyl-cardiolipin) and sterol (cholesterol, or ergosterol) patterns of phase separation in liposomes in the absence and presence of polyphenols were investigated. We demonstrated that the phase segregation scenario is determined by the hydrophobic mismatch between ordered and disordered domains. Polyphenols depressed the phase segregation of membranes composed of SM, Chol, and POPC (or DOPC). Another ternary lipid mixtures tested were virtually insensitive to the introduction of modifiers. It was suggested that polyphenols affected the hydrophobic mismatch through the fluidization of the ordered and disordered domains. Ability of the modifier to immerse into membrane and to fluidize the domains depended on the hydrophobicity of the modifier molecules, geometric shape of domain-forming lipids, and their packing density. The study was supported by RFBR (16-04-00806) and SP-69.2015.4.

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– 23. Biomimetic structures and systems –

P-714

Membrane curvature induction by curved DNA-based scaffoldsH. G. Franquelim¹, A. Khmelinskaia^{1,3}, J.-P. Sobczak², H. Dietz², P. Schwille¹¹Max Planck Institute of Biochemistry, Martinsried, Germany; ²Technical University of Munich, Garching, Germany; ³Graduate School of Quantitative Biosciences, Ludwig-Maximilians-University, Munich, Germany

Curved peripheral membrane protein scaffolds, such as Bin/Amphiphysin/Rvs (BAR) domain proteins, are capable of inducing and stabilizing curvature on cellular membranes. By emulating the characteristic banana-shapes of BAR domains using DNA origami, we developed synthetic biomimetic scaffolds of different degrees of curvature. The curved DNA-based nanostructures were then functionalized with cholesterol anchors and their interaction with lipid model membranes studied using biophysical techniques. We show that positioning and number of membrane anchors are essential to ensure effective binding of the curved DNA-based scaffolds to lipid bilayers. Distinctive membrane deformations, such as tubulation, could be triggered at increased scaffold membrane densities, and the shape of those deformations correlated with the intrinsic curvature of membrane-bound nanoscaffolds. Taken together, our approach reveals a minimal set of modules and preconditions required for shape-dependent membrane curvature generation by artificial scaffolding elements, such as degree of curvature, membrane affinity and surface density.

P-716

24:1 and 16:0 sphingolipids in fluid membranes containing cholesterolA. B. García-Arribas^{1,2}, E. J. González-Ramírez^{1,2}, J. Sot^{1,2}, I. Areso^{1,2}, A. Alonso^{1,2}, F. M. Goñi^{1,2}¹Biofisika Institute (UPV/EHU, CSIC), Spain; ²Departamento de Bioquímica y Biología Molecular (UPV/EHU), Spain

The effects of C24:1 sphingolipids have been tested in phospholipid bilayers containing cholesterol. Confocal microscopy, DSC, and AFM techniques have been used. More precisely, the effects of C24:1 ceramide (nCer) were evaluated and compared to those of C16:0 ceramide (pCer) in bilayers composed basically of dioleoyl phosphatidylcholine, sphingomyelin (either C24:1, nSM or C16:0, pSM) and cholesterol. Combination of equimolecular amounts of C24:1 and C16:0 sphingolipids were also studied. Results show that both pCer and nCer are capable of forming segregated gel domains. Force spectroscopy data point to nCer having a lower stiffening effect than pCer, while the presence of nSM reduces the stiffness. DSC reveals T_m reduction by nSM in every case. Furthermore, pSM seems to better accommodate both ceramides in a single phase of intermediate properties, while nSM partial accommodation of ceramides generates different gel phases with higher stiffnesses caused by inter-ceramide cooperation. If both pSM and nSM are present, a clear preference of both ceramides towards pSM is observed. These findings show the sharp increase in complexity when membranes exhibit different sphingolipids of varying N-acyl chains, which should be a common issue in an actual cell membrane environment.

P-715

Glycerolipidyl-cyclodextrins: self-aggregation studies and interactions with model membranesA. L. Furlan¹, S. Buchoux¹, Y. Miao², V. Banchet³, J. Michel³, V. Bonnet², C. Sarazin¹¹Génie Enzymatique et Cellulaire FRE CNRS 3580, Université de Picardie Jules Verne, 80000 Amiens Cedex, France; ²Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources, UMR CNRS 7378, Université Picardie Jules Verne, 80000 Amiens Cedex, France; ³Plate-forme d'Imagerie Cellulaire et Tissulaire (PICT), Université de Reims Champagne Ardennes, 51685 Reims Cedex 2, France

Supramolecular assemblies of nonionic amphiphilic cyclodextrins represent quite promising nanoparticles to encapsulate drugs with interesting hemocompatibilities compared some hydrogels. Because they are both highly biocompatible and biorecyclable parts, glycerolipidyl-cyclodextrins constitute a solid challenge for biomedical nanoparticles. The self-assembling properties of two amphiphilic glycerolipidyl-cyclodextrins, DMCD and DOCD, exhibit micromolar critical aggregation concentration (CAC). Additionally, the stability of the aggregates obtained were studied revealing that nanoparticles made of DOCD can be stored at 4°C for prolonged period without any issue. Furthermore, a sound characterization of these nanoparticles was performed by combining DLS, cryo-TEM and MD simulations. The interaction with target membrane models have been studied by ³¹P and ²H NMR on MLV with different composition. ³¹P NMR experiment shows a global insertion of amphiphilic cyclodextrins and ²H NMR data suggest disturbance of the lipid dynamics due to cyclodextrins.

P-717

The lipid bilayer: news from the insideB. Gironi¹, A. Lapini², M. Paolantoni¹, A. Morresi¹, P. Foggi^{1,2}, P. Sassi¹¹Dipartimento di Chemistry Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy; ²LENS, European Laboratory for Nonlinear Spectroscopy, Via N. Carrara 1, Sesto Fiorentino, Firenze, Italy

The solubility of small molecules into a lipid bilayer is central to many biological and biotechnological processes. Lieb and Stein [1] proposed that small molecules dissolve and diffuse by “hopping” between empty voids and molecular dynamics studies have shown that the largest voids are located in the center of the membrane, that they increase by increasing temperature and unsaturations in the acyl chains, decrease by adding cholesterol, and that it depends on lipid composition [2]. Unfortunately, this description can hardly find an experimental confirmation and the diffusion and permeation routes at the atomic level are still not completely understood. In the present study we use IR spectroscopy, both stationary and time resolved, to characterize the properties of cholesterol loaded POPC liposomes. To this extent we use a vibrational probe, hexacarbonyl tungsten W(CO)₆, to follow the solubility of a small hydrophobic molecule inside the membrane at different temperatures and solvent composition (DMSO/water solutions at different DMSO mole fraction). [1] Lieb W. R. and Stein W. D., Nature 224, 1969, 240 – 243 [2] Möller M.N. *et al.*, Biochim. et Biophys. Acta 1858, 2016, 2923–2930

Posters

– 23. Biomimetic structures and systems –

P-718

Formation of lipid-bilayer nanodiscs by styrene/maleic acid (2:1) copolymerA. Grethen¹, A. Oluwole Olusegun^{1,2}, B. Danielczak¹, C. Vargas¹, S. Keller¹¹Molecular Biophysics, University of Kaiserslautern, Kaiserslautern, Germany; ²Department of Chemistry, University of Ibadan, Ibadan, Nigeria

Styrene/maleic acid (SMA)¹ and diisobutylene/maleic acid (DIBMA)² copolymers self-insert into lipid membranes to form polymer-bounded nanodiscs. This membrane mimic enables structural and functional characterization of membrane proteins in a native-like nanoscale environment. SMA exists in forms with various styrene/maleic acid ratios, which affects its solubilization behavior. SMA(2:1) has emerged as the standard polymer for nanodisc formation as it displays the highest efficiency in extracting membrane proteins.³ We systematically characterized the solubilization properties of SMA(2:1) by monitoring the formation of nanodiscs from lipid vesicles using calorimetry, spectroscopy, and light scattering. Phase diagrams thus obtained were compared with those of other amphiphilic copolymers, thereby revealing that SMA(2:1) is the most efficient solubilizer. Particle size distributions showed that pH, ionic strength, and lipid composition influence the solubilization efficiency. These findings pave the way for the judicious use of amphiphilic copolymers for solubilizing and stabilizing membrane proteins in native nanodiscs.

[1] Knowles et al. *J. Am. Chem. Soc.* **2009**, 131, 7484[2] Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, 56, 1919[3] Morrison et al. *Biochem. J.* **2016**, 473, 4349

P-720

3D structure of chiral alpha-peptoids by means of experimental and theoretical circular dichroismF. Jolibois¹, O. Roy³, L. Perrin², G. Dumonteil³, C. Taillefumier³¹LPCNO, Université de Toulouse, CNRS, INSA, UPS, France; ²ICBMS UMR 5246, Université de Lyon, France;³Université Clermont Auvergne, CNRS, SIGMA Clermont, Institut de Chimie de Clermont-Ferrand, France

Peptoids (N-substituted glycine oligomers) hold a special place in the foldamer field. On the one hand, they are very close mimics of peptides because their main chain is entirely composed of glycine units. On the other hand, the peptoids main chain is deprived of N–H donors and chirality, two main features that control peptide folding. In order to reintroduce chirality properties, side chains with asymmetric carbons can be appended to the nitrogen atoms of the backbone. In this context, peptoid oligomers with tert-butylethyl (tbe) side chains have been synthesized up to the nonamer size. Among different characterisation techniques, circular dichroism (CD) spectra have been recorded in acetonitrile solvent and exhibit specific features when the size of the oligomer increases. In order to interpret such evolution, quantum chemical calculations using TD-DFT approach and Polarizable Continuum Model for solvent modelling have been performed on monomer to hexamer compounds. Direct comparison with experimental spectra allowed to fully characterized the three dimensional structure of these foldamers and to highlight unambiguous specific intramolecular interactions responsible of the CD signature.

P-719

DNA-templated peptide assemblyJ. Jin¹, E. G. Baker², J. Bath¹, D. N. Woolfson^{2,3}, A. J. Turberfield¹¹Department of Physics, University of Oxford, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, UK; ²School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK; ³School of Biochemistry, University of Bristol, Biomedical Sciences Building, University Walk, Bristol, BS8 1TD, UK

The rules that relate amino acid sequence to the structure and function of peptides and proteins are not well understood. By contrast, the assembly of DNA nanostructures relies on simple and well-defined base-pairing rules, but straightforward assembly comes at the expense of limited functionality. We combine the strengths of the two classes of biomolecule to use well-defined DNA templates to control the assembly and function of peptides, using a pair of heterodimeric coiled-coil peptides as a model system. Peptides are conjugated to unique DNA tags *via* copper-free Huisgen cycloaddition chemistry. Hybridization of the DNA tag to complementary targets on DNA nanostructures allows control over their identity, number and arrangement. We find that assembly of DNA nanostructures decorated with one heterodimer peptide each is consistent with the formation of the heterodimeric coiled-coil at the interface. The distribution of assemblies in the EM provides a novel way to calculate the Kd for this peptide-DNA system. This test model shows we can accurately guide and control peptide assembly using DNA templates.

P-721

A DNA origami-based single-molecule assay for multidentate protein-pharmacophore bindingC. Kieler¹, F. V. Reddavid², S. Tubbenhauer¹, G. Grundmeier¹, Y. Zhang², A. Keller¹¹Technical and Macromolecular Chemistry, University of Paderborn, Germany; ²B CUBE - Center for Molecular Bioengineering, TU Dresden, Germany

Recently, precise functionalization of DNA origami with functional entities such as proteins by chemically modified staple stands has been widely used, particularly in single molecule studies. Here, we use a DNA origami-based single molecule assay to study the binding of the proteins streptavidin (SAv), alpha-1-acid glycoprotein (AGP), and the protease trypsin. We demonstrate the feasibility of monodentate and bidentate binding of SAv to iBt on DNA origami. SAv-iBt binding is investigated in dependence of pH, incubation time. We then extend this approach to study multidentate binding of SAv, AGP, and the protease trypsin in parallel, and evaluate the effect trypsin-catalyzed protein digestion on the binding yields for AGP and SAv. Binding affinities are determined by atomic force microscopy. By extending the present approach to other medically relevant protein-ligand systems and proteases, the detailed investigation of the effect of the geometric arrangement of multiple pharmacophores on their inhibitory effect becomes possible. The so obtained data will be relevant for the chemical reformatting of individual pharmacophores into a single drug molecule without loss of affinity or selectivity.

Posters

– 23. Biomimetic structures and systems –

P-722

Superstructure-dependent non-intercalative drug binding to DNA origami nanostructuresF. Kollmann¹, S. Ramakrishnan¹, M. A. Kostianen², G. Grundmeier¹, V. Linko², A. Keller¹¹Technical and Macromolecular Chemistry, Paderborn University, Paderborn, Germany; ²Biohybrid Materials, Department of Bioproducts and Biosystems, Aalto University, Aalto, Finland

DNA origami nanostructures are widely investigated with regard to their applicability in fields as diverse as nanoelectronics, molecular sensing, and drug delivery. For the latter application, drug loading of the DNA origami delivery systems is typically achieved via intercalation between the base pairs of the DNA double helices. By employing DNA origami nanostructures with deliberately underwound double helices, intercalator loading can be tuned.

Here, we investigate the binding of the drug methylene blue (MB) to different 2D and 3D DNA origami nanostructures. MB has been used extensively as a therapeutic agent to treat numerous diseases, including malaria and methemoglobinemia, and is currently investigated as a potential photosensitizer for photodynamic therapy. Furthermore, MB may interact with DNA via intercalation, groove binding, and electrostatic interactions. Using UV-Vis spectroscopy, we observe non-intercalative binding of MB to the DNA origami, with the measured dissociation constants depending on DNA origami superstructure. Our results may contribute to the design of DNA origami-based drug carriers with tailored loading and release properties.

P-724

Biophysical characterization of lipopeptides involved in membrane fusionA. Koukalová¹, Š. Pokorná¹, N. L. Mora², A. L. Boyle², R. Šachl¹, M. Hof¹, A. Kros²¹J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Czech Republic; ²Leiden Institute of Chemistry, Leiden University, Netherlands

Membrane fusion is a key process naturally occurring in cells as it facilitates e.g. delivery of chemicals across biological barriers to specific cellular locations. Intracellular fusion in vivo is triggered by a specific interaction of SNARE protein complex forming coiled-coil bundles. Designing of an efficient and specific system that might be useful for in vivo applications, e.g. direct drug delivery into cytosol, requires a good understanding of molecular mechanism behind the fusion event. The present study aims to contribute to the current knowledge by advanced fluorescent microscopic techniques. We use a reduced system in which fusion-related proteins are replaced by two complementary synthetic lipopeptides consisting of peptides K4[(KIAALKE)4] or E4[(EIAALEK)4], a PEG linker and a cholesterol anchor. Model lipid membranes are used as biophysical models of the plasma membrane. Our interest is to study the interaction of K/E peptide with lipid membranes and examine their aggregation behavior. The data show that peptide K tends to aggregate and interact with the membrane more strongly than peptide E, which might decrease the efficiency of membrane fusion. Their behavior, however, heavily depends on the membrane lipid composition.

P-723

The interaction between amyloid- β peptides and model membranes containing cholesterol and melatoninT. Kondela¹, B. Demé², N. Kučerka^{1,3}¹Faculty of Pharmacy, Comenius University in Bratislava, Slovakia; ²Institut Laue-Langevin, Grenoble, France;³Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, Russian Federation

Alzheimer's disease (AD) is a devastating neurodegenerative disease caused by the formation of senile plaques, primarily consisting of amyloid-beta (Ab) peptides. The crucial role in this process is imparted by peptide-membrane interactions, changing the structural properties of membrane. These changes are known to be modulated also by membrane composition. In particular, cholesterol increases the order of lipid hydrocarbon chains and increases the stiffness of membrane. On the other hand, melatonin increases the fluidity of membrane. Our previous experiments [Drolle et al., BBA 2013] revealed the counteracting effect of melatonin to that of cholesterol in neat lipid membranes. We have extended our investigations recently by including transmembrane Ab peptide in these model membranes. Small angle neutron diffraction measured at four different contrast conditions was utilized for an unambiguous determination of structure in transversal direction. The obtained bilayer structure reflected the elevated amounts of cholesterol by its thickening, while the fluidizing effect of melatonin evoked the membrane thinning. Results of our experiments possibly confirm the melatonin's potential role in preventing the development of AD.

P-725

A comparative study on the fusion kinetics of different SNARE familiesS. Krüger¹, P. Halder², R. Jahn², C. Steinem¹¹Georg-August-University, Germany; ²Max-Panck-Institute for biophysical Chemistry, Germany

Membrane fusion is a fundamental process in every living cell. For the sorting of different cargoes in the organism a highly regulated sequence of vesicle docking and merging of two bilayers is required. Therefore, different model systems have been developed to study SNARE (soluble NSF-attachment receptor proteins)-mediated fusion focusing on the neuronal SNARE-system (Syntaxin-1a, SNAP25, VAMP-2). In recent years further SNARE families have been characterized, among them the late-endosomal SNAREs (Syntaxin-7 and 8, vti1B and VAMP-8).

To ensure fusogenicity of the neuronal system, a stabilized complex (Δ N49) with a soluble fragment of the vesicle-SNARE Synaptobrevin-2 was developed. Anisotropic measurements showed that this fragment might hinder the kinetics of fusion drastically, since the removal and the final zippering of the tetrameric complex takes around 5 sec. In contrast endosomal SNARE-proteins form a tetrameric coiled-coil-complex with each protein donating one SNARE-motif and one transmembrane domain to the complex.

Using bulk and single vesicle fusion assays like pore spanning membranes, we study the differences in membrane fusion within both SNARE-families. In theory, the new SNARE-family allows for different topologies as well as faster fusion kinetics.

Posters

– 23. Biomimetic structures and systems –

P-726

Antimicrobial peptide-lipid interactions and lateral diffusion in model membranes via ^{31}P CODEX NMRA. Lai^{1,2}, P. M. MacDonald^{1,2}¹University of Toronto, Canada; ²University of Toronto Mississauga, Canada

Lateral diffusion of phospholipids is a process essential to membrane function, and its accurate determination can provide insights into kinetics of membrane-associated biochemical reactions. Here we describe the application of CODEX (Centerband-Only Detection of Exchange) and our powder-average model to measure lateral diffusion of phospholipids in lipid bilayers. CODEX is an ideal experiment for these systems because ^{31}P NMR can be measured in natural abundance, eliminating the need for synthetic labels.

The plasma membrane in many types of bacteria contains neutral and anionic lipids. The charge arises from differing phosphate head groups, which will appear as separate narrow resonances in a ^{31}P CODEX spectrum. Unhindered, lipids will experience free diffusion. Many antimicrobial peptides selectively bind to anionic lipids, causing them to diffuse at a slower rate than the unbound lipids. By comparing differences in diffusion rates between the lipids bound to the peptide of interest versus the bulk, we will gain insights into the mechanisms governing the efficacy of antimicrobial peptides in disrupting the bilayer. We investigated the binding between the cationic peptide polylysine and the model antimicrobial peptide KL14 with LUV consisting of the neutral lipid POPC and anionic lipids POPG, DOPA, DOPS, and cardiolipin.

P-728

Biomimetic microreactor-based strategy for studying NO-Synthase activitiesP. Lefrançois¹, J. Santolini², S. Arbault¹

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NO-Synthases (NOS) are the only source of nitric oxide (NO^\bullet) in mammals. These enzymes produce NO^\bullet but also diverse Reactive Oxygen and Nitrogen Species (ROS and RNS), according to different enzymatic mechanisms. These are not well understood because NOS activities are usually studied in bulk and steady-state conditions. To decipher more efficiently on NOS mechanisms, it is mandatory to keep the enzyme in active state and biologically-related scales (volume, time). The project aims to develop biomimetic microreactors to characterize NOS reactional pathways via *in situ* detection of produced species by electrochemistry and fluorescence microscopy. This approach is indeed compatible with the time-scales of diffusion and reactivity of ROS-RNS, allowing their characterization when generated by the enzyme. Furthermore, a vesicles' network is used to trigger the enzymatic reaction in a controlled manner. Pseudo-selective fluorescent probes for ROS-RNS inform on the localization and diffusion of species. In addition, by placing a sensitive ultramicroelectrode at the vicinity of the microreactor, true fluxes of species generated by NOS (NO^\bullet , NO_2^- and H_2O_2) under various activation/inhibition conditions can be quantified with this micrometric approach.

P-727

Mesoscale structure of lipid monolayers mimicking red blood cell membranesB.-D. Lechner¹, J. L. Trick², C. D. Lorenz², P. Winlove¹, P. G. Petrov¹

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The mechanical properties of the red blood cells are a major determinant of their function.¹ The mesoscopic (microdomain) organisation of the outer leaflet of the erythrocyte plasma membrane has been a subject to a number of studies,² however, little is known about existence and composition of microdomains in the inner leaflet. This is especially important in disease (e.g. diabetes) where this structure may be compromised due to oxidative and other chemical stresses.³

Here we investigate the microdomain structure of synthetic lipid monolayers mimicking the composition of the inner and outer leaflet of RBC plasma membranes by means of the Langmuir trough technique coupled with fluorescence microscopy. The impact of the replacement of cholesterol by 7-keto-cholesterol, a main oxidation product in the plasma membrane, on the membrane microdomain structure is also studied.

The lateral organization of the monolayers is compared to atomistic Molecular Dynamics simulations of the same lipid monolayers to identify the lipid species responsible for the mesoscale ordering of the monolayer.

1. H.W.G. Lim *et al.* *PNAS* **2002**, 99, 16766.
2. K. Simons *et al.* *Annu. Rev. Biophys. Biomol. Struct.* **2004**, 33, 269.
3. C. Mokken *et al.* *Annals. Haematol.* **1992**, 64, 113.

P-729

Immersion depths of lipid carbons in bicelles measured by paramagnetic relaxation enhancement

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Myriads of biological processes occur in or at lipid membranes. Knowledge about the localization of proteins, lipids, and other molecules within biological membranes is thus crucial for the understanding of such processes. Here, we present a method to determine the immersion depths of lipid carbon atoms in membranes by paramagnetic relaxation enhancement (PRE). As membrane mimetics we employ small isotropic bicelles made of synthetic lipids and of natural *Escherichia coli* phospholipid extract. PREs were measured in the presence of different nitroxide labeled lipids with the radical located in the head group and at different positions in the acyl chain. Theoretical PREs were calculated using the Solomon-Bloembergen equations and immersion depths of the lipid carbon atoms were obtained by a least-square fit of the theoretical to the experimental PREs. The carbon immersion depths correspond well to results obtained by other methods. Differences do not exceed 3–5 Å. This means that the method presented here provides sufficient resolution to distinguish the localization of carbons in different regions of the lipid bilayer. The approach presented here may be extended to the determination of protein localization in membranes employing realistic membrane mimetics like bicelles made of *E. coli* phospholipid extract.

Posters

– 23. Biomimetic structures and systems –

P-730

Revealing cardiolipins influence in the construction of a significant mitochondrial membrane model

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Cardiolipins constitute a class of lipids that is highly variable *in vivo*, both in percentage and acyl chains, but are essential for the maintenance of mitochondria integrity and dynamics. For this reason we chose to use a natural extract of a cardiolipin (CL) mixture, commercially available, of bovine heart. Using a natural extract of CL the study gains closeness to the influence of the “cardiolipins” in a mitochondrial membrane model. Different lipid mixtures of the three main cardiac ventricular mitochondrial membrane constituent phospholipids: phosphatidylethanolamine (PE), phosphatidylcholine (PC), and CL were studied. In order to determine their thermotropic properties steady-state fluorescence anisotropy, dynamic light scattering (DLS), and Nuclear Magnetic Resonance (NMR) were used. The main transition temperature obtained, similar for the different lipidic systems, is CL dependent. Moreover CL seems to be able to act as fluidizing or rigidifying agent, depending the characteristics of main membrane matrix component. In summary, CL presence or absence in a mitochondrial membrane model dramatically changes membrane's properties and therefore its inclusion in mitochondrial model system membranes should always be considered.

P-732

Novel Cholesterol like compounds in cellular approachesA. L. Matos¹, D. Grill¹, L. Rakers², F. Glorius², V. Gerke¹¹Institut für Medizinische Biochemie - WWU, Germany;²Organisch-Chemisches Institut - WWU, Germany

Cholesterol is an essential component in cellular membranes which is not only important for regulation of membrane fluidity, but also participates in several membrane trafficking and signaling events. Here we present a new synthetic analogue of cholesterol that incorporates into cellular membranes and can be labeled with fluorescent dyes. The compound is based on N-heterocyclic carbene (NHC) that are heterocyclic rings composed of a carbene and minimum one nitrogen atom in the structure (Bourissou et al 2000). The corresponding imidazolium salts containing 1,3 dialkyl groups have been studied for their capability of mimicking lipid properties in artificial membranes (Drücker et al 2016). This promising area carries the potential of in situ functionalization, modifying properties of the structure (Hopkinson et al 2014). In our study we investigate cholesterol like structures containing imidazolium salts regarding their behavior in cell membranes. As cholesterol is an essential molecule in biological membranes, our objective is to study how this related structure behaves in cellular membranes using different types of cells and microscopy analysis.

Bourissou, D. et al, 2000. *Chemical Reviews*.Drücker, P., et al, 2016. *Langmuir*.Hopkinson, M.N., et al, 2014. *Nature*.

P-731

Elucidation of membrane protein-membrane interactions in polymer vesiclesA. F. Mason^{1,2}, P. Thordarson¹¹School of Chemistry, UNSW, the Australian Centre for Nanomedicine ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Australia; ²Technische Universiteit Eindhoven, Netherlands

Polymer vesicles, or polymersomes, provide a thermodynamically stable platform for the investigation of several important natural phenomenon, including compartmentalization and the incorporation of transmembrane proteins. However, polymersome membranes are typically much thicker than their natural lipid counterparts, which leads to their superior stability. In addition, polymersomes often have a chemically homogeneous membrane, in contrast to cell and organelle membranes, which have different inner and outer leaflets.

In this work, a family of diblock copolymers were synthesized and mixed in specific ratios to generate polymersomes with asymmetric membranes, with poly(ethylene glycol) on the outer leaflet and poly(acrylic acid) on the inner leaflet. Cytochrome *c* oxidase (CcO), a protein found in the inner membrane of the mitochondria, was subsequently inserted and was found to have a templating effect on the structure of the polymer membrane. The membrane asymmetry, thickness, and the ability to form vesicles were all influenced strongly by the presence of CcO. These results provide valuable insights into the role membrane proteins might have on membrane structure, paving the way for more complex protein-polymer systems with applications in synthetic cell research.

P-733

Recyclable bionanostructures from self-assembling peptides

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Protein self assembly is of interest in a variety of natural systems from the amyloid plaques found in Alzheimer's disease to protein polymers involved in membrane remodelling. For an organism to function correctly, it is vital that these polymers can be recycled into their individual monomer units. In nature, this is achieved by the use of a class of proteins known as AAA⁺ ATPases, which generate mechanical force to release subunits from large protein complexes.

Inspired by these natural systems, self assembly proteins have been investigated as a material for a range of different applications including scaffolds for regenerative medicine, drug delivery and even electronics and data storage. In general, the range of functionality and bottom up assembly process makes them highly appealing as a nano-material. This talk will discuss how we can create specific nano-structures from self-assembly proteins, which can be recycled back to their original constituent monomers upon the addition of an ATPase, and the potential applications of this.

Posters

– 23. Biomimetic structures and systems –

P-734

Corneocyte structure and molecular mobility: effect of hydration

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The skin is the largest organ of human body and protects us from desiccation and uptake of chemicals from the external environment. The outermost horny layer of the skin epidermis - the stratum corneum (SC) serves as the main barrier and consists of multilayered stack of lipid matrix in which the corneocytes are embedded. The molecular structure and functional properties of SC is largely affected by hydration. To investigate the nature of hydration induced changes in the SC at different relative humidities (RH), we performed small angle X-ray diffraction and solid-state nuclear magnetic resonance experiments on SC and isolated corneocytes. We show that hydration at different RH leads to a change in the inter-chain distance of keratin filaments present within the corneocyte. A gradual increase in the molecular dynamics of specific amino acids that are present in the protruding terminals of keratin filament is observed when the level of hydration increases. These changes in the structure and dynamics are evident when the hydration level reaches a threshold limit which lies around 85 % RH. These molecular changes can be directly related to changes in SC macroscopic properties, as evident through an abrupt increase in water swelling of both SC and corneocytes at the same RH.

P-736

Pore-spanning membranes: a tool to study single vesicle content release in SNARE driven fusionP. Mühlenbrock, R. Hubrich, J. Kuhlmann, L. Schwenen
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The transmission of electrical signals between neurons in the nervous system is a fast and highly regulated process in which fusion of highly curved synaptic vesicles with the planar presynaptic membrane leads to neurotransmitter release into the synaptic cleft. This way of neuronal signal transmission is driven by so-called SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein receptor) proteins.

To understand the mechanisms of this process a unique *in vitro* fusion assay has been established that enables the detection of individual fusion events. Pore-spanning membranes (PSM) on closed cavities serve as a model system that mimics the planar geometry and the tension of the presynaptic membrane. SNARE-mediated fusion of highly curved unilamellar vesicles with the target membrane allows to monitor the release of the vesicle content into the enclosed aqueous compartment in a sensitivity of single fusion events. Spinning disc confocal microscopy (SDCM) is used to record the whole process from vesicle-docking to content release with high time resolution.

P-735

Generation of complex self-assembled DNA microstructures inside water-in-oil microdropletsM. Morita^{1,2}, S.-I. M. Nomura³, S. Murata³, M. Yanagisawa⁴, M. Takinoue¹¹Dept. Comp. Sci., Tokyo Tech., Japan; ²Biomed. Res. Inst., Natl. Inst. Adv. Ind. Sci. Tech. (AIST), Japan; ³Dept. Robotics, Tohoku Univ., Japan; ⁴Dept. Appl. Phys., Tokyo Univ. Agric. Tech., Japan

The generation of complex self-organized structures inspired by nature is one of the major goals of material science. Based on bottom-up DNA nanotechnology, various-sized DNA structures such as nanoscopic DNA origami and macroscopic DNA hydrogels have been created to date. Mesoscopic DNA structures have great potential for future technologies such as molecular robotics and biomedical application. However, concepts are still missing to facilitate the production of mesoscopic self-assembled DNA structures. Here, We present DNA microstructures that are kinetically controlled to assemble complex microstructures, and the investigation of its formation mechanism. The DNA microstructures were consisted of self-assembly of Y-branched DNA monomers (Y-DNA) at the interface of a water-in-oil (W/O) microdroplet covered with a cationic lipid. The electrostatic interaction between the lipid and the DNA induced gelation of Y-DNA on the interface of the W/O microdroplets. We found that the kinetic process of the Y-DNA self-assembly brought the DNA gel in a fractal-like pattern at the interface of the W/O microdroplets, i.e., complex DNA microstructures. We hope that this generation method can lead to novel way for construction of functional DNA microstructures.

P-737

A DNA-based sensor for macromolecular crowdingC. U. Murade¹, G. T. Shubeita^{1,2}¹New York University Abu Dhabi, UAE; ²The University of Texas at Austin, USA

The interior of all living cells is a highly crowded environment, where biomolecules typically occupy 30% to 40% of the total cell volume. This is very different from the sparse *in vitro* environment where biomolecular function is often studied. In a crowded environment, physical effects can lead to altered association rates of biomolecules, altered diffusion rates, and altered macromolecular conformation, all of which can lead to altered protein function. While examples of enzymatic rates increasing by three orders of magnitude in an artificially crowded environments have been reported, it remains unclear how general this is and how crowded the microenvironments within cells where these enzymes natively function. To begin to understand this problem it is important to quantify the degree of crowding within the cell and its various sub compartments. To that end, we have developed a DNA-based macromolecular crowding sensor that reports on the degree of crowding by changing its conformation. Experiments conducted in artificially crowded solutions containing Polyethylene Glycol (PEG) or Ficoll-70 demonstrate the sensitivity and versatility of this sensor.

Posters

– 23. Biomimetic structures and systems –

P-738

Solubilization of membrane proteins into functional lipid-bilayer nanodiscs by new polymersA. O. Oluwole^{1,2}, B. Danielczak¹, A. Meister³, J. O. Babalola², C. Vargas¹, S. Keller¹¹Molecular Biophysics, University of Kaiserslautern, Germany; ²Department of Chemistry, University of Ibadan, Ibadan, Nigeria; ³Institute of Chemistry and Institute of Biochemistry and Biotechnology, Martin Luther University Halle–Wittenberg, Germany

Once removed from their natural environment, membrane proteins depend on membrane-mimetic systems to retain their native structures and functions. To this end, lipid-bilayer nanodiscs that are bounded by styrene/maleic acid (SMA) copolymers have been introduced as alternatives to liposomes for *in vitro* membrane-protein research.¹ Here, we introduce an alternating diisobutylene/ maleic acid (DIBMA) copolymer that shows equal performance to SMA in solubilizing phospholipids, stabilizes an integral membrane enzyme in functional bilayer nanodiscs, and extracts proteins of various sizes directly from cellular membranes. Unlike aromatic SMA, aliphatic DIBMA has only a mild effect on lipid acyl-chain order and dynamics, does not interfere with optical spectroscopy in the far-UV range, and does not precipitate in the presence of low millimolar concentrations of divalent cations.² Thus, lipid-bilayer nanodiscs surrounded by DIBMA enable *in vitro* investigations of membrane proteins in a native-like nanoscale environment that is amenable to a broad range of biophysical methods.

¹ Knowles et al. *J. Am. Chem. Soc.* **2009**, *131*, 7484² Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919

P-740

Structural properties of oxidized membranes investigated by Small Angle X-ray Scattering (SAXS)R. D. Rosa¹, F. Spinozzi², R. Itri¹¹Institute of Physics - University of Sao Paulo, Brazil;²Department of Life Sciences, Università Politecnica delle Marche, Ancona, Italy

Lipid oxidation impacts on biological membranes inducing changes in the membrane physical properties which may lead to cell physiology malfunction. Some oxidized lipids have hydrophilic groups pendant on the hydrocarbon chains, as hydroperoxidized groups for instance, some on shortened acyl oxidized lipid chains. Such oxidized lipid can remain in the membrane, and add to already complex composition of membranes. In this work, we are particularly interested in comprehending how lipid chemical transformations induced by oxidative stress can alter membrane structural features and, by turn, membrane-protein interaction. In this way, SAXS results from liposomes representing model lipid vesicles composed of different amounts of unsaturated, oxidized and saturated lipids will be presented and discussed. Interestingly, the analysis of SAXS data allows us having details on liposome structure as well as localizing the oxidized species inside the vesicle lipid bilayer. Furthermore, the self-assembling of amyloid-like proteins on liposomes surface can also be investigated by SAXS, pointing out the importance of lipid composition that may play a role in protein aggregation. The authors thank FAPESP and CNPq for financial support.

P-739

Influence of pH on the therapeutic effects of Artepillin C: enhancing its use as an anticancer drugW. M. Pazin¹, A. S. Ito², C. J. L. Constantino¹¹Faculdade de Ciências e Tecnologia - UNESP, Brazil;²Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - USP, Brazil

Artepillin C is the major constituent among all the compounds identified in the Brazilian green propolis, displaying antitumor, anti-inflammatory and antioxidant activities. A previous study has shown that the compound, due to its particular structure, interacts with amphiphilic aggregates formed of phospholipids, which may be an important step to elicit its biological action. This study shows the interaction of Artepillin C with model membranes in systems that mimic the acidic extracellular matrix (E_m) of healthy and tumor cells. Langmuir monolayer experiments confirm the preference of the compound for amphiphilic environment, independent of its protonation state. Despite, under environment with lower pH, which mimics E_m of tumor cells, Artepillin C increases the fluidity of the model membrane, confirmed by changes in the compressibility modulus value (C_s^{-1}), which could not be observed for the system of healthy E_m . The results evidence that the compound has potential to change and damage only the structure of tumor cell membranes, while for healthy model systems, Artepillin C could only act in benefit of the cells, acting for instance against lipid peroxidation without damaging the membrane. Acknowledgements: FAPESP (2016/09633-4) and CNPq (304981/2012-5).

P-741

Following kinetic processes of membrane proteins in real time using fluorescence microscopy

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We have employed rapid fusion of oppositely charged liposomes to deliver separately reconstituted membrane proteins (MPs) into a common lipid bilayer, containing different proteins. With this technique, we functionally co-reconstituted different oxidases and ATP synthase from *E. coli* into unilamellar vesicles. Successful fusion was confirmed by measuring redox driven ATP synthesis. Furthermore, we have used the same methodology to reconstitute MPs into giant unilamellar vesicles (GUVs) by fusion of small positively charged proteoliposomes with negatively charged GUVs. With the help of functional assays and fluorescence microscopy, we have confirmed the functional incorporation of three different MPs into the GUV membrane.

Next, we aim to measure transmembrane transport processes of MPs in GUVs directly under the fluorescence microscope. In comparison to small liposomes, GUVs do not suffer from disadvantages like high membrane curvature and small interior volume (concentrations change quickly in a 50 nm liposome). Furthermore, GUVs are large enough to be monitored by fluorescence microscopy.

Here, we present our current efforts to *in situ* monitor proton pumping events of MP complexes like the ATP synthase or cytochrome *c* oxidase employing soluble, lipid anchored pH sensitive probes.

Posters

– 23. Biomimetic structures and systems –

P-742

Mimicking the cellular cortex: Artificial F-actin networks physiologically attached to lipid bilayersM. Schön¹, H. Nöding², I. P. Mey¹, A. Janshoff², C. Steinem¹¹Institute of Organic and Biomolecular Chemistry, University of Göttingen, Germany; ²Institute of Physical Chemistry, University of Göttingen, Germany

The shape, mechanical stability and driving forces of cells are highly dependent on their cytoskeleton. Filamentous actin (F-actin) is one of the main proteins which contributes to this biological network. In this work an artificial actin cortex is associated to a lipid bilayer via the linker protein Ezrin. Ezrin is N-terminal bound to PIP₂ lipids and provides a C-terminal actin binding domain. This linkage is used to achieve a physiological way to attach the network. It's pinning points can be varied by changing the surface coverage of Ezrin on the lipid bilayer. With raising the receptor lipid's (PIP₂) molar ratio inside the bilayer the surface coverage of Ezrin is increased leading to an increased pinning point density.

The self-organization and the mechanics of networks attached to bilayers with different pinning point density were characterized. Therefore the height and mesh size were evaluated via fluorescence microscopy. Furthermore, active and passive microrheology was used to obtain frequency dependent viscoelastic properties of various dense networks. This macroscopic network response was subsequently used to determine microscopic transient crosslink contribution. This led to information about the Ezrin-PIP₂ binding kinetics.

P-744

Simulation and experimental design of synthetic protein based motorsL. Small¹, A. Cano-Marques¹, M. Zuckermann², N. Forde², S. Cobb¹, E. Bromley¹¹Durham University, UK; ²Simon Fraser University, Canada

Improving our understanding of biological motors, both to fully comprehend their activities in vital processes, and to exploit their impressive abilities for use in bionanotechnology, is highly desirable. One means of understanding these systems is through the production of synthetic protein based molecular motors.

Here we discuss the simulation and attempts to construct and characterise two protein based motors. The first, the tumbleweed, is a three footed motor designed to move via ligand gated diffusion along a DNA track. The second, the bar motor, is a two footed motor in which processivity is produced via a conformational switch in the structure of the motor.

Simulation data for both of these systems will be presented along with the current status of experiments to realise both motors. In particular we will discuss the design and production of conformational switching in a bar motor precursor using the photo-sensitive cis-trans isomerization of azobenzene.

P-743

Pore spanning membranes as a versatile biomimetic tool to study phase separation and lipid diffusionJ. Sibold¹, V. Tewaag¹, S. Spindler², N. Teske¹, I. Mey¹, V. Sandoghdar², C. Steinem¹¹Georg-August University, Göttingen, Germany; ²Max Planck Institute for the Science of Light, Erlangen, Germany

Membrane nanodomains, often also termed lipid rafts, are known to provide functional areas within biological membranes. They are highly dynamic and the size of membrane nanodomains is typically in the range of only 50-100 nm making the observation of these domains highly challenging.

In artificial membrane systems, raft domains are frequently mimicked by using liquid-ordered (*l_o*)/liquid disordered (*l_d*) phase separated bilayers. We show that pore spanning membranes (PSMs) are a well-suited system to analyze *l_o*/*l_d* phase separation. By means of fluorescence microscopy, we were able to observe phase separation in the free standing and the supported membrane areas. The obtained results are discussed in the framework of different adhesion energies of the membrane on the supported and freestanding areas. Furthermore, the PSMs provide insight into the diffusion behavior of single lipids dependent on the lipid-substrate interaction. By means of interferometric scattering microscopy (iSCAT), where a gold-nano-particle is attached to a lipid and followed by single particle tracking, we were able to monitor the diffusion behavior of individual lipids in the free-standing and supported areas of the PSMs.

P-745

Lipid membranes on elastic substrates

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The choice of support for supported lipid bilayers is often limited to a passive and unyielding material such as mica or silica. In nature lipid membranes are supported by dynamically remodelling structures such as the basal lamina, the actin cortex or the extracellular matrix. For this reason we have developed a model system in which we couple lipid patches of cellular dimensions to a flexible polymer surface, the area of which can be reversibly controlled.

We find that lipid membranes on deformable substrates exhibit several distinct mechanisms of stress relaxation. They can 1) follow the substrate's area changes by absorbing and expelling lipid protrusions; 2) open and close micron-size circular pores; or 3) slide over the expanding/contracting substrate. We show that these responses are determined by the nature of the substrate-membrane coupling and the lubricating properties of the interstitial fluid. Switching between the regimes is easily controlled through changes to solution pH and/or substrate hydrophobicity.

Our work holds promise for the design of the next generation of lipid-based surface coatings that may change shape, exhibit controllable permeability or reveal underlying structures upon changes in the environmental conditions.

Posters

– 23. Biomimetic structures and systems –

P-746

Investigation of biomimetic membrane models with the ENTH domain of epsin1 and substrate surfaces

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Biomimetic membrane models such as giant unilamellar vesicles (GUVs) and pore-spanning membranes (PSMs) are well established for the investigation of structural and functional properties as well as membrane-protein interactions. Within these models different characteristics like lipid composition, enclosed compartment volume or membrane tension can be varied to elucidate on specific interactions.

While adhered GUVs (aGUVs) offer large enclosed compartments, huge membrane areas and allow the tuning of membrane tension, PSMs also offer the advantage of solid supported and free-standing membrane areas with decreased compartment volume and free standing membrane areas.

Employing aGUVs, we were able to study the effects of the epsin N-terminal homology (ENTH), a protein domain involved in the clathrin mediated endocytosis. Treatment of aGUVs with ENTH results in changes of membrane shape due to specific receptor lipid binding and protein insertion driven by osmotic pressure and membrane tension. PSMs are better suited to study diffusion barriers and protein clustering of ENTH-membrane interactions. To overcome the major limitation of PSMs, the absent signal on the pore rims, we changed the surface chemistry resulting in low membrane tension and observable fluorescence on the whole PSM.

P-748

A new microfluidic device for creating biomimetic tissue-like structures

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Giant unilamellar vesicles (GUVs) are excellent biomimetic systems. Typical experiments involve dilute suspensions where there is little or no contact between vesicles. In nature, however, cells of multicellular organisms are packed together and rarely isolated for the entire duration of their life cycle. To address this experimental gap, we aimed to better mimic this natural environment by designing a microfluidic system that can create biomimetic tissue-like structures. The design consists of a series of micro-structured posts which hydrodynamically captures multiple GUVs at specific spatial locations, thus creating a tissue-like assembly. We demonstrate that molecules can be homogeneously added/removed in less than 30 s and adjacent micro-posts can increase the trapping efficiency by 97%. The device also filters the GUVs to trap a specific size range of vesicle depending on the channel height. The entire device contains 204 traps with an average of 79 GUVs per trap, totalling over 16,000 GUVs per device. Therefore, it can be used for ligand-membrane or permeation studies where monitoring multiple GUVs is advantageous for statistical analysis. This work is part of the MaxSynBio consortium which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society.

P-747

Surfactant fluorination finds its way in membrane-protein research

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Since fluorinated surfactants stabilize membrane proteins but are considered incapable of solubilizing them directly, they are usually employed only after initial extraction using conventional detergents. Using ¹⁹F and ³¹P NMR, fluorescence anisotropy decays, light scattering, and calorimetry, we could demonstrate that fluorinated octyl maltoside (F₆OM) interacts with and solubilizes lipid vesicles similar to conventional detergents, but without compromising membrane order.¹ We found that F₆OM chaperones the functional refolding of a membrane enzyme to yield functional proteoliposomes. F₆OM and a hydrogenated maltoside detergent mix within a single type of micelle regardless of the mixing ratio, whereas combining F₆OM with a hydrocarbon surfactant bearing a zwitterionic phosphocholine headgroup gives rise to two coexisting micelle populations at high mole fractions of F₆OM.² This paves the way for using two-component surfactant mixtures for extracting and stabilizing membrane proteins *in vitro*.

¹Frotscher et al. *Angew. Chem. Int. Ed.* **2015**, 54, 5069

²Frotscher et al. *Anal. Chem.* **2017**, 89, 3245

Posters

– 24. Drug discovery and delivery –

P-749 (O-142)

Intrinsic vs. observed thermodynamic and kinetic parameters of carbonic anhydrase-ligand interactionV. Linkuviene¹, V. O. Talibov², H. Danielson^{2,3}, D. Matulis¹¹Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Lithuania;²Department of Chemistry - BMC, Uppsala University, Sweden;³Science for Life Laboratory, Uppsala University, Sweden

Drug design is often based on a structure-activity relationship analysis. Different groups of a compound can show various binding affinity and association-dissociation rates to a target protein. However, the observed protein-ligand binding parameters do not reveal the influence of other contributing reactions in this interplay.

We use carbonic anhydrase (CA), vital enzymes that catalyze carbon dioxide hydration. But misregulation of their expression can cause diseases, such as epilepsy, cancer, etc. Therefore CA inhibitors are of interest in pharmaceutical research, but since CAs have highly similar structure of the active site, it is difficult to design compounds that would selectively inhibit one isoform.

CA – compound interaction was determined by the fluorescent thermal shift assay, isothermal titration calorimetry, and surface plasmon resonance. Combination of parameters, which can be obtained by three methods, provided the direction of optimization of the compound binding affinity and selectivity towards the desired CA isoform.

P-751 (O-144)

Anti-transferrin receptor antibody conjugated PLGA nanoparticles for temozolomide deliveryM. J. Ramalho¹, J. A. Loureiro¹, J. Lima², M. A. N. Coelho¹, M. C. Pereira¹¹LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Portugal; ²Cancer Signaling and Metabolism Group, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

The classical temozolomide (TMZ) treatment for glioblastoma is rarely curative, due to TMZ's poor ability to cross the blood-brain barrier (BBB) [1]. Thus, improved delivery systems may be a suitable strategy to overcome those limitations. TMZ was encapsulated in poly(lactide-coglycolide) nanoparticles (PLGA NPs), and dual-targeting approach was envisaged using NPs modified with anti-transferrin receptor monoclonal antibody (mAb), since this receptor is overexpressed in BBB and cancer cells [2]. The PLGA NPs showed mean diameters of 200 nm and encapsulation efficiency of 45%. Release studies showed a lower diffusion rate of TMZ from mAb-PLGA NPs when comparing with non-modified NPs. The mAb layer on the surface of PLGA NPs may hinder water permeation, resulting in a lower diffusion. The cytotoxicity of TMZ-PLGA NPs was evaluated on two human glioma cell lines, U251 and U87, demonstrating that the antineoplastic effect of TMZ is enhanced by the nanoformulation. However, cytotoxic effect of TMZ was to some extent decreased with the mAb-modification, due to the lower release rate of the drug. Further studies will confirm whether the use of the mAbs will present advantages in *in vivo* conditions, allowing increased transport across the BBB.

P-750 (O-143)

Development and characterization of polymeric nanoparticleB. F. C. Patricio¹, J. M. Pietra², C. Martinez², S. D. V. Alonso², T. Barboza³, S. A. Lopes³, P. M. Bisch¹, G. Weissmüller¹¹Laboratório de Física Biológica, IBCCF, CCS, UFRJ, Brazil; ²Laboratório de Biomembranas, UNQ, Argentina;³Departamento de radiologia, HU-UFRJ, Brazil

Nanotechnology aim to solve several pharmacological problems. To guarantee the success of nanoparticles (NP) it is important to ensure homogenous particle size, surface hydrophilicity and prolonged circulation time. This work aimed to produce and evaluate PLA/PVA and PEG/PLA/PVA NP with different size and surface properties and evaluated it by *in vivo* test. The NP were produced by double emulsion method with changes in both power and sonication time to obtain NP with different sizes. Using the Zebrafish WT model (CE-UNQ 2/2014) it was possible to observe that the PLA/PVA NP were more cardiotoxic than those pegylated. Furthermore, hepatotoxicity and neurotoxicity were higher in large pegylated NP. Finally, both NP were labeled with ^{99m}Tc and injected into Wistar rats (CEUA 086/15). It was obtained gamma camera images and the organs activity were measured. It was possible to define that pegylation decreased the uptake of the larger NP by the liver, but do not change the uptake of the smaller ones. The non-pegylated NP accumulated in the lungs. In this work, it was possible to determine the better conditions for production of NP. Also, it allows the understanding of the toxicity and biodistribution of the nanoparticles at *in vivo* models.

P-752

Stimuli-responsive gelatin nanoparticles for treating corneal infections/inflammation

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Corneal infections/inflammation (keratitis) is a major cause of visual impairment in the world. An important issue in managing keratitis is the administration of two drugs (anti-inflammatory and anti-microbial), in an appropriate balance, over prolonged periods. However, the various anatomical and physiological barriers that normally protect the eye create obstacles during therapeutic interventions.

To overcome this, in the present study we demonstrate a nanostructure that exploits two key features of the keratitis patho-physiology (over-expressed TLRs and increased protease activity) to modulate drug activity. The nanostructure consists of a ketoconazole (ket)-loaded gelatin core with surface conjugated anti-TLR4 antibodies. While the anti-TLR4 antibodies assist in corneal adhesion and suppressing inflammation, the gelatin core degrades in response to the proteases providing an on-demand ket release. The nanoparticles were successfully tested on cell culture models, and showed a significant increase in corneal retention, a decrease in inflammation and pathogen load, in rat models of keratitis.

The present study addresses basic issues in corneal drug delivery and demonstrates a smart drug-delivery system for an effective management of keratitis.

Posters

– 24. Drug discovery and delivery –

P-753

Super-resolution STED investigation of membrane receptors as drug targetsB. Angelov¹, A. Angelova²¹Institute of Physics, ELI Beamlines, Academy of Sciences of the Czech Republic, Na Slovance 2, CZ-18221 Prague, Czech Republic; ²Institut Galien Paris-Sud, CNRS UMR 8612, Univ. Paris-Sud 11, Université Paris-Saclay, LabEx LERMIT, F-92296 Châtenay-Malabry, France

Membrane receptor targeting by ligands depends on the receptor topology, oligomerization, and domain formation. Here the nanoscale organization of the tropomyosin-related kinase receptor type B (TrkB), a promising therapeutic target for Alzheimer's (AD) and Huntington's (HD) diseases, amyotrophic lateral sclerosis, epilepsy, and severe neuropsychiatric disorders (schizophrenia, anxiety, and bipolar disorder), is studied by super-resolution stimulated emission depletion (STED) microscopy in membranes of differentiated human neuroblastoma SH-SY5Y cells representing stages of neuronal damage and survival. The performed imaging and subdiffraction quantification of TrkB receptor oligomerization suggests that membrane receptor clustering may impede specific ligand- and drug-receptor binding, and thus diminish the molecular recognition by neurotransmitters acting as drugs. We show that ligand administration together with polyunsaturated docosahexaenoic acid (DHA) modulates the sizes of the protein clusters in the lipid membranes. Perspective treatment of severe neurological diseases may require disruption of the receptor clusters in order to enable high affinity neurotrophic protein pharmaceuticals to exert efficient therapeutic action.

P-755

Nanostructured photosensitizers tailored to trigger photo-induced regulated cell death

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In Photodynamic Therapy (PDT) synthetic photosensitizers (PS) and light are used to efficiently induce photosensitized reactions in specific tissues. We aim to increase the efficiency of PS by tailoring them to execute damage in well defined cellular targets and consequently to induce specific mechanisms of regulated cell death. Several nanostructured PS will be used to alter cell localization and intracellular release of the PS including functionalized nanosilica, metal-based PS and PS adsorbed in biopolymers. Examples will be shown in which small damages in cytoplasmic membrane cause necrotic cell death, damages in mitochondria cause mainly apoptotic cell death, while parallel damages in mitochondria and in lysosome cause autophagic cell death. We will also show how a polymer made of poly-lysine modified with protoporphyrin IX can be used to modulate cell adhesion to substrates. We propose to discuss the development of nanostructured and target-specific PS aiming to improve the efficiency of PDT protocols against cancer and infection diseases.

P-754

Characterizing drug resistance of cancer cells with single-cell Raman spectroscopy

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Drug resistance of human gastric carcinoma cells (BGC823) was investigated using single-cell Raman spectroscopy (RS). Normal BGC823 and drug-resistant BGC823 cells (DR-BGC823) which cultured with continuous short-term exposure to paclitaxel (PTX), were collected and analyzed. Time-dependent effects for 48 hour treatments with different concentrations of PTX for normal and DR-BGC823 cells were evaluated. RS intensity changes with PTX over time produced dissimilar results for the two types of cells. Average RS intensities of normal BGC823 cells initially decreased and then increased with PTX treatment after 24 hours. In contrast, upon exposure to PTX, the average intensity of DR-BGC823 cells initially increased until 12 hours, then gradually decreased and remained steady. Additionally, heterogeneous characteristics of drug resistant cells arising from normal BGC823 cells were observed and quantified by the coefficient of variation (CV). Our study indicates that single-cell RS can quantitatively evaluate cell-to-cell differences in drug resistance, and further suggests that RS may be useful in systematically characterizing drug resistance of cancer cells at the single-cells level in the future.

P-756

Subcellular localization of anionophore derivatives indicated as replacing drugs of defective CFTRD. Baroni¹, M. Fiore¹, R. Quesada², O. Moran¹¹Istituto di Biofisica, CNR, Italy; ²Departamento de Química, Facultad de Ciencias, Universidad de Burgos, Spain

Cystic fibrosis (CF) is a lethal autosomal recessive genetic disease that originates from the defective function of the CFTR protein, a cAMP-dependent chloride channel involved in fluid transport across epithelia. Thanks to their capability to replace the ion transport independently from the genetic mutation that affects the CFTR, small synthetic transmembrane anion transporters, named anionophores, candidate as new potential CF therapeutics. With the aim to evaluate both their impact on cell physiology and their "drugability", we started to analyse their subcellular localization exploiting their intrinsic fluorescence or the introduction of a fluorescent fluorophore into their structure. Confocal microscopy, conducted on CHO and HEK cells shows that anionophores distribute initially throughout the cells, but predominantly in the neighbourhood of the plasma membrane. Interestingly, with time, their distribution into the plasma membrane and other membrane-bound cell compartments becomes more evident, confirming their lipophilic nature. Cell fractionation followed by mass spectrometry analysis corroborate these data.

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Posters

– 24. Drug discovery and delivery –

P-757

Magnetic nanoparticle mediated gene transfer to induce apoptosis in cancer cellsH. Basoglu¹, B. Goncu², F. Akkas³

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CD95 (Fas) is a complex surface protein which can trigger apoptosis when interacted with its ligand CD95L (FasL). However CD95 doesn't induce apoptosis in cancer cells, because of the changes in death domain (DD) of CD95 (eg. procaspase-8). In this study pDNA which can express both CD95 and GFP, was transfected to MCF-7 cells via magnetic nanoparticle (MagNP) and lipid based transfection reagent. Then expression of DD of CD95 and stimulation of apoptosis were investigated. Magnetofection method was used to MagNP mediated pDNA transfer and the transfection efficiency was compared. Western blot was performed to investigate DD of CD95. Effect of FasL on transfected cells were analyzed. According to manufacturer's suggestion 100ng pDNA/well were used for lipid based transfection whereas 200ng pDNA/well with MagNP bring out better transfection result. MCF-7 cells express no procaspase-8 however transfected cells do. Lipid based transfection reduces β -actin and lamin-B1 protein expression. Transfection of CD95-GFP tagged pDNA significantly increase apoptosis. FasL interaction indicated slight increment of apoptosis in transfected cells. MagNP mediated gene transfer is very efficient for transfection and does not reduce other proteins.

P-759

Understanding drug interactions at the cell membraneH. M. Britt, J. A. Mosely, J. M. Sanderson
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Understanding interactions between pharmaceutical drug candidates and biological components is a vital part of the complex task of drug development. Such interactions have the ability to influence the activity, pharmacokinetics and side effects of the drug candidate, and thus its success as a pharmaceutical. Phospholipids, as major components of both the cell membrane and drug delivery liposomes, are one such biological component with which interactions must be considered. Using *in vitro* models and *in vivo* cellular systems, a novel reaction between phospholipids and cationic amphiphilic drug molecules has been identified. This reaction, termed intrinsic lipidation, results in formation of a modified drug molecule and increased levels of lyso-lipid. Intrinsic lipidation reaction products are found to modify drug activity, induce drug side effects including phospholipidosis, and to alter membrane composition and structure. Further, the structure and functionalities of drug candidates have been shown to greatly impact the propensity of a drug molecule towards the reaction. Intrinsic lipidation offers increased insight into drug interactions, their causes, and their implications, a topic of vital importance in tackling the modern day challenges of drug design.

P-758

Preclinical Cytotoxicity Investigations in Stem Cells with an “All Inclusive” ApproachA. Bazzzone, C. Weichbrodt, S. Stölzle-Feix, P. Mumm, N. Becker, U. Thomas, K. Juhasz, L. Doerr, M. Beckler, C. Haarmann, M. George, A. Brüggemann, N. Fertig
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Human stem cell-derived cardiomyocytes (hiPSC-CMs) have recently proven to recapitulate key features of human cardiac electrophysiology *in vitro*. Chip-based approaches allow parallel patch clamp recordings without compromising data quality or technical sophistication. We present mid to high-throughput voltage and current clamp recordings of Nanion's automated patch clamp devices. Since drug efficacies may vary with temperature, we present recordings at room and at physiological temperatures. In addition to patch-clamping experiments, we present hybrid impedance (cell contractility) with MEA-like extracellular field potential (EFP) recordings on Nanion's CardioExcyte96. Experiments were complemented with optical stimulation of monolayers of hiPSC-CMs expressing the light-gated cation channel Channelrhodopsin2. This approach allows frequency-dependent drug screening and detection of potential side effects on Na^+ , Ca^{2+} and repolarizing K^+ channels. Cytotoxic responses of cell monolayers involve metabolic or biochemical changes that affect the morphology of the cells, or reduce their overall viability. In that regard, effects of a number of reference compounds tested for long-term cytotoxicity in hepatocyte-like cells (i.e. Paracetamol), will be presented.

P-760

ASADOCK: a web server for compound screening that aggregates biochemical assay results

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Compound screening methodologies are widely used in the pharmaceutical industry in the initial steps of drug development. However, most methodologies consider only *in silico* results from docking algorithms and other approaches. Here we present ASADock: Asynchronous Screening based on Assays and Docking, a novel approach to compound screening that aggregates biochemical assay results that were realized with the compounds present in its database. The database contains more than a million compounds extracted from the Drugs for Neglected Diseases initiative published results. Together with their 3D structure already resolved and any biochemical assay available. The results contain binding free energy and biochemical information like IC50 and Potency. We also provide links to more detailed information on each individual assay. Blind docking as well as localized docking can be used for screening the compounds. The user can also select pre-filtered groups of compounds that have assays targeting distinct species of microorganisms. We are optimizing the software to run in a multi-scale grid-like server architecture inside the High Performance Computational National System – SINAPAD, where it will be made available free for the international Scientific Community.

Posters

– 24. Drug discovery and delivery –

P-761

Explore novel JNK3 ligand by HTS of designed scaffold library using FTS assayC. Cheng^{1,2}, X. Gao², N. Pharm³, R. J. Quinn³, Z. Xiao¹, Z. Liu⁴¹Dept. of Anatomy and Developmental Biology, Monash University, Clayton, Melbourne, Australia; ²National Lab. of Macromolecules, Inst. of Biophysics, Chinese Academy of Sciences, Beijing, China; ³The Eskitis Institute, Griffith University, Brisbane, Australia; ⁴The iHuman Institute, ShanghaiTech University, Shanghai, China

The c-Jun N-terminal Kinases is evolutionary highly conserved serine/threonine kinases. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 has much more limited expression confined to the nervous system. Numerous findings suggested that JNK3 involves in the pathogenesis in neurodegenerative disease, such as AD and PD. Tremendous medicinal chemistry efforts leading to a large number of JNK inhibitors, even some showed reasonable *in vitro* profiles. However, due to the highly conserved ATP-binding site among JNKs and other MAPKs members, the highly isoform-selective JNK3 inhibitors are extremely rare. In this study, a chemically diversified lead-like scaffolds library up to 2024 pure compounds was constructed, synthesized and assay-ready formatted. The library was applied high throughput screening by using fluorescence thermal shift assay to identify JNK3 binding scaffolds. Totally eight compounds (classified as five scaffold groups) well fit Lipinski's Rule-of-five indicating a high probability of drug-like characters from the view of drug discovery. The binding geometries and interactions were visualized by docking small molecules into JNK3 crystal structure. We believe that this insight analysis could be potentially used for selective JNK3 inhibitors development.

P-763

Albumin coated nanoparticles of 2-oxazoline based copolymer enhances bioavailability of curcuminS. Datta^{1,1}, A. Jutkova^{1,2}, J. Kronek^{1,3}, V. Huntosova^{1,1}, D. Jancura^{1,2}, P. Miskovsky^{1,1}¹Center for Interdisciplinary Biosciences, Safarik University, Kosice, Slovakia; ²Department of Biophysics, Safarik University, Kosice, Slovakia; ³Institute of Polymers, Slovak Academy of Sciences, Bratislava, Slovakia

Tunable properties and biocompatibility of poly (2-oxazoline) based nanoparticles make them ideal for the biological application. Here we report on the synthesis and use of new copolymeric nanoparticles based on 2-oxazolines for the targeted delivery of curcumin. The nanoparticles prepared by two different methods (dialysis and dropwise method of aggregation) from a newly synthesized block-copolymers are highly stable for more than 3 weeks. Both types of nanoparticles with nanomolar formulation showed high loading capacity for a well-known anticancer agent curcumin. Encapsulation of curcumin inside these spherical nanoparticles improves its solubility and makes stable for more than 3 weeks. Nanoparticles were also coated with human serum albumin to increase their circulation time and to target cancerous cells. 60–80 nm size of these nanoparticles make them suitable for cancer targeting because of the EPR effect. The targeting efficiency of curcumin loaded albumins coated nanoparticles was evaluated using several types of cell lines (MCF7, U 87 and HeLa cells). These nanoparticles were superior to the conventional polymeric nanoparticles in its size, formulation, stability and biocompatibility. This work was supported by the grant APVV–15-0485 of the Slovak Research and Development Agency.

P-762

Small molecule photosensitisers for light-activated cell deathD. R. Chisholm¹, T. Pallet¹, C. Holden¹, C. Newstead¹, R. Manning¹, J. M. Girkin¹, C. A. Ambler¹, R. Valentine², E. Pohl¹, A. Whiting¹¹Durham University, UK; ²High Force Research Ltd., UK

Localised production of reactive oxygen species (ROS) is a defence strategy employed in both animal and plant systems in response to pathogens. However, the release of high levels of ROS also causes rapid, non-selective death of animal, plant and bacterial cells. Photodynamic therapy (PDT) exploits this by using photosensitisers that respond to specific wavelengths of light to generate large amounts of ROS that destroy diseased tissues. However, most existing photosensitisers used for PDT are not 'typical' drug compounds, and suffer from drawbacks including long biological half-life and high molecular weight, and also cause skin photosensitivity for weeks after treatment due to off-target effects. We have developed two drug-like photosensitisers, DC324 and DC473, which cause rapid cell death when activated with UV or violet light. DC324 elicits effective, non-selective cell death in epithelial and fibroblastic cells. DC473 possesses a terminal alkyne moiety, which allows for 'click' conjugation with azide-functionalised biomolecules. DC473 can be conjugated to targeting antibodies, allowing for rapid, selective destruction of a specific cell type. The powerful destructive action of DC324 and DC473 could have wide-ranging applications in PDT, wound cleansing, and antimicrobials.

P-764

Docking-based virtual screening for potential activity against bacterial pyruvate kinaseC. Ergun¹, E. D. Akten², P. Doruker¹¹Department of Chemical Engineering and Polymer Research Center, Bogazici University, Bebek, 34342, Istanbul, Turkey;²Department of Bioinformatics and Genetics, Kadir Has University, Fatih, 34083, Istanbul, Turkey

Pyruvate kinase (PK) catalyzes the last step of glycolysis; therefore, it is an essential protein for almost all living organisms. It has been identified as a hub protein with different allosteric mechanisms in humans and bacteria, which makes pyruvate kinase an attractive antibacterial drug target. In this study, docking-based virtual screening is performed for pyruvate kinase of methicillin-resistant *Staphylococcus aureus* (MRSA) using a database of known drugs. The screening focuses on a binding pocket at the interface of the tetrameric enzyme, to which several inhibitors have been shown to bind selectively in MRSA over humans (Axerio-Cilies et al., ACS Chemical Biology, Vol 7, 2011). Flexible ligand docking was carried out using Autodock v4 for over 1900 known drugs and an inhibitor IS-130 as a control (Morris, et al., J. Comp. Chem., Vol 16, 2009). Two cardiovascular drugs, Oxyfedrine HCl and Propranolol HCl, were identified as top-scoring molecules, among which the former is known to have antibacterial effects (Mazumdar, et al., Biol. Pharm. Bull, Vol 28, 2005). Further research needs to be conducted to determine whether these two drugs affect the pyruvate kinase activity of MRSA and other antibiotic-resistant bacteria.

Posters

– 24. Drug discovery and delivery –

P-765

Structure - biological activity relationship of estrogenic flavonoids from *Plantago sempervirens*A. D. Farcas^{1,2}, L. Buimăgă-Iarinca¹, A. C. Mot^{1,2}, V. A. Toma^{1,2}, M. Parvu²¹National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania;²Babes-Bolyai University, Cluj-Napoca, Romania

Plantago species have high antioxidant potential due to their great amounts of polyphenolic compounds, such as luteolin, apigenin and rutin, known under the name of flavonoids. The chemical structure of these compounds is suitable for a mechanism that relies on the conventional hydrogen-donating antioxidants, but may also exert modulatory actions in different signaling pathways. Another antioxidant strategy of reducing oxidative stress may rely on their potential of activating the estrogen receptor- β (ER β) which is an essential mechanism for many diseases prevention.

This study focuses on determining the positive interactions among estrogenic flavonoids, most relevant in *P. sempervirens* and estrogen receptor- β using molecular docking. In addition, the estrogenic activity of this biomolecules was investigated using *in vivo* models exposed to oxidative stress, in which the hormonal level and plasma indices of the oxidant status were evaluated.

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P-767

Dynamic equilibrium of Aurora-A kinase activation loop revealed by single molecule spectroscopyJ. A. H. Gilbert¹, H. Sarkar¹, P. Sheldrake², J. Blagg², L. Ying¹, C. A. Dodson¹¹National Heart & Lung Institute, Imperial College London, UK; ²Cancer Research UK Cancer Therapeutics Unit, Institute of Cancer Research, UK

The conformation of the activation loop (T-loop) of protein kinases underlies enzymatic activity and influences the binding of small molecule inhibitors. We have used single molecule fluorescence spectroscopy to monitor the movement of the T-loop (activation loop) of Aurora-A kinase between two major conformations. Phosphorylated Aurora-A is in dynamic equilibrium between a DFG-in-like active T-loop conformation and a DFG-out-like inactive T-loop conformation and we have measured the rate constants of interconversion. We have directly measured the equilibrium between the two conformations and determined the free energy difference between them. Addition of activating protein (TPX2) shifts the equilibrium towards the active T-loop conformation, whereas addition of the inhibitors MLN8054 and CD532 favours an inactive T-loop conformation. Notably, 36% of Aurora-A still occupies an active T-loop conformation in the presence of saturating CD532. We show that TPX2 and MLN8054, whose binding modes are thought to be mutually exclusive, bind Aurora-A simultaneously. Our approach will enable conformation-specific effects to be integrated into inhibitor discovery across the kinome and we outline some immediate consequences for structure-based drug discovery.

P-766

Nanoparticles and the blood-brain barriers in vitro: Carriers as well as barrier modulatorsH.-J. Galla¹, S. Cramer¹, R. G. Rempe¹, R. Quiao², M. Gao²¹Institute for Biochemistry University of Münster, Germany; ²Institute of Chemistry, Chinese Academy of Science, Beijing, China

The main obstacle for the treatment of brain diseases is the restriction of the passage of pharmaceuticals across the blood-brain barrier. Endothelial cells line up the cerebral microvessels and prevent the uncontrolled transfer of polar substances by intercellular tight junctions. In addition to this physical barrier, active transporters of the multi-drug-resistance act prevent the passage of hydrophobic substances. This paper will introduce an *in vitro* porcine brain derived cell culture system that closely resembles the BBB *in vivo*. The aim of the present work is to develop strategies based on the use of nanoparticles to overcome the BBB. This could either be reached by a reversible opening of the barrier or by a targeted transport facilitated for example by nanoparticles. It will be shown that nanoparticles can cross the blood brain barrier by two mechanisms: a) opening the tight junctions and thus allowing parallel import of substances into the brain; b) receptor mediated endocytosis using brain specific target molecules. Possible applications are drug transfer as well as transfer of contrast agents for magnetic resonance imaging (MRI). Impedance spectroscopy will be introduced as a valuable tool to investigate barrier properties.

P-768

Improving Crystals by Collaboration - Innovation from Molecular Dimensions

J. M. B. Gordon, C. E. Naylor, J. Hobbs

Molecular Dimensions Ltd, UK

Molecular Dimensions has a history of collaborating with leading structural biologists to develop novel research tools in the field of protein crystallography. In this presentation, we will illustrate how to improve your chances of growing, identifying and mounting diffraction-quality crystals using our range of innovative products.

Our novel stability and sparse matrix screens improve your chances of getting an initial hit. However, it is easy to miss small crystals hidden in precipitate, especially when viewing hundreds of trial conditions: our UVEX imaging systems and laboratory microscopes make the task easier. CryoProtX™ gives you cryocooling with confidence. It's range of mixes balance cryoprotection and protein stabilization so your crystal doesn't crack or dissolve. LCP crystallisation is an important technique for membrane protein structure determination. But the viscous drops it creates can make mounting crystals with a traditional loop extremely challenging. With the DiffraX™ thin-film sandwich plate, a single drop is easily cut from the ultrathin films with a scalpel or scissors. Their small volume means they can then be placed directly in our adapted cryomounts for cryocooling by plunging in liquid nitrogen.

Posters

– 24. Drug discovery and delivery –

P-769

miR-300 Regulates DNA Damage Response Induced by Ionizing Radiation in Human Lung CancerJ. He¹, W. Wei¹, X. Feng^{1,2}, J. Hua¹, Z. Lu³, J. Wang¹¹Institute of Modern Physics, Chinese Academy of Sciences, China; ²School of Pharmacy, Lanzhou University, China; ³Gansu university of Chinese medicine, China

Previous studies revealed that miR-300 was involved in the cellular response to IR or chemotherapy drug. However, whether miR-300 could regulate the DNA damage responses induced by extrinsic genotoxic stress in human lung cancer and the underlying mechanism remains unknown. In this study, the effects of miR-300 on DNA damage repair, cell cycle arrest and apoptosis induced by IR were investigated in human cell lung cancer cell lines. It was found that ectopic expression of miR-300 by transfection with miR-300 mimics not only greatly enhanced the cellular DNA damage repair ability, but substantially abrogated the G2 cell cycle arrest and apoptosis induced by IR in A549 and Glc82 cells. Bioinformatic analysis predicted that p53 was a potential target of miR-300, and the luciferase reporter assay showed that miR-300 significantly suppressed the luciferase activity through binding to the 3'-UTR of *p53* mRNA. In addition, over-expression of miR-300 in A549 cells significantly reduced IR-induced p53 expression. Flow cytometry analysis and colony formation assay showed that miR-300 desensitized A549 cells to IR by suppressing p53-dependent G2 cell cycle arrest and senescence. These data demonstrate that miR-300 regulates the DNA damage response to IR through targeting p53 in lung cancer cells.

P-771

Fine control of cell populations heterogeneity and its impact on GPCRs pharmacological studiesJ. Holm, R. W. Hansen, L. Bojarskaite, K. L. Martinez
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Proteins are expressed over a wide range of concentrations in distinct tissues and within different cells in the same tissue. This physiological heterogeneity of cell populations is hypothesized to be exploited to fine tune the cell response to external stimuli but remains poorly investigated.

We made an extensive characterization of heterologous protein expression profiles in mammalian cell cultures. These quantitative single cell studies reveal how classical methods used for heterologous expression of proteins in mammalian cells (e.g transient transfection, stable cell lines (mono- and polyclonal), inducible expression vectors) impact the heterogeneity of the cell population to be studied.

Using a prototypical G Protein Coupled Receptor, which is the main drug target superfamily today, we show by controlling protein expression levels in cells combined with quantitative studies at the single cell and ensemble levels, that the receptor density in cells has an effect on its signaling (i.e. cAMP signaling, interaction with cytosolic proteins and internalization).

P-770

Pulmonary surfactant and drug delivery: sharing an interfacial tripA. Hidalgo¹, C. García-Mouton¹, F. Salis², G. Orellana², J. Pérez-Gil¹, A. Cruz¹¹Department of Biochemistry, Faculty of Biology, Complutense University, Madrid, Spain; ²Department of Organic Chemistry, Faculty of Chemistry, Complutense University, Madrid, Spain

The respiratory surface of lungs is coated by a lipid-protein material, pulmonary surfactant (PS). Alveoli are subjected to compression-expansion cycling and PS prevents pulmonary collapse during breathing. PS adsorbs very rapidly into the air-liquid interface and spread efficiently along it. Cycling forces the interfacial films to fold into three-dimensional structures that exclude certain lipid/protein complexes and non-compressible molecules from the interface, including drugs or nanocarriers.

This work has evaluated how PS travels along the interface, whether the spreading affects the structure of PS films, what happens if it shares the trip with drugs or particles, and whether the vehiculated entities are excluded from the interface under cycling. We have developed a new setup that connects traditional Wilhelmy and Langmuir-Blodgett troughs by interfacial bridges. PS samples travel along the interface from donor compartments, which act as reservoirs, to recipient chambers. Here, PS films are subjected to compression-expansion cycles, and the behaviour of films is interpreted in terms of molecular flows through the interface and structural 2D-3D transitions. A model is presented to interpret the possible diffusion of PS/drug combinations into the lungs.

P-772

Targeting DnaG using NMR-fragment based virtual screening for the design of antibacterial agentsS. Ilic, B. Tam, M. Singh, S. R. Akabayov, B. Akabayov
Ben-Gurion University of the Negev, Israel

The DNA replication process in *Mycobacterium tuberculosis* (*Mtb*) is a promising but underexploited target for the development of novel antibiotics.

We have developed an approach to identify inhibitors for *Mtb* DnaG primase, which is a key enzyme in the DNA replication machinery of *Mtb*. For the development process we have used DNA primase from bacteriophage T7. T7 primase has several structural features that are similar to bacterial (including *Mtb*) primases, making it an ideal model to study bacterial primases.

Using NMR screening, fragment molecules that bind T7 primase were identified and then exploited in virtual filtration to select larger molecules from a virtual library. The molecules were docked to the primase active site using the available T7 primase crystal structure and ranked based on their binding energies to identify the best candidates for functional and structural investigations. Biochemical assays revealed that some of the molecules inhibit T7 primase-dependent DNA replication. The binding mechanism was delineated via NMR spectroscopy. Importantly, some of the molecules inhibit also the activity of DnaG primase of *Mtb*. Our studies yielded new class of antituberculous agents and provide new tools for fragment-based lead discovery.

Posters

– 24. Drug discovery and delivery –

P-773

Complex formation between photosensitizer hypericin and high-density lipoproteins (HDL)A. Jutkova¹, L. Blascakova¹, J. Kronek³, P. Miskovsky^{1,2}, D. Jancura^{1,2}¹Department of Biophysics, Faculty of Science, P. J. Safarik University, Jesenna 5, 041 54 Kosice, Slovakia; ²Center for Interdisciplinary Biosciences, P. J. Safarik University, Jesenna 5, 041 54 Kosice, Slovakia; ³Polymer Institute of the Slovak Academy of the Sciences, Dubravska cesta 9, 845 41 Bratislava 45, Slovakia

Cancer cells require cholesterol to achieve an increased rate of growth. It has been shown that high-density lipoproteins (HDL) are involved in cholesterol transport in some malignancies, including breast, ovarian and prostate cancer. HDL molecules can be used as a delivery system for targeted drug biodistribution to the tumor cells as they are biodegradable, do not trigger immune response and the incorporation of a drug into the HDL does not affect the drug stability. The main task of this work was to construct a HDL-based drug delivery system and to investigate properties of HDL complex with a photosensitizer hypericin (Hyp) by means of fluorescence spectroscopy. With the aim to increase the efficiency of Hyp transport, the HDL molecules were coated by dextran (Dex) and modified dextran (Dm). We have found that the coating of the HDL with Dm results in a 38 % reduction of Hyp redistribution from the complex HDL/Dm to free HDL molecules. We can conclude that HDL coating with Dm can effectively prevents the redistribution of incorporated molecules of Hyp in HDL to other serum lipoproteins. This work was supported by APVV-15-0485 grant.

P-775

H-ferritin–doxorubicin targets and kills tumorsM. Liang¹, K. Fan¹, X. Yan¹¹Institute of Biophysics, Chinese Academy of Sciences, China; ²Institute of Biophysics, Chinese Academy of Sciences, China; ³Institute of Biophysics, Chinese Academy of Sciences, China

Here, we show that natural H-ferritin (HFn) nanocages can carry high doses of doxorubicin (Dox) for tumor-specific targeting and killing without any targeting ligand functionalization or property modulation. The HFn-Dox specifically bound to transferrin receptor which is biomarker for many tumors and exhibited more than 10-fold higher intratumoral drug concentration than free Dox and significantly inhibited tumor growth after a single-dose injection. Importantly, HFn-Dox displayed an excellent safety profile that and it can be easily produced with high purity and yield. Thus, these unique properties make the HFn nanocage an ideal vehicle for efficient anticancer drug delivery.

P-774

Magnetic Stable Isotopes as New Trend in Medical BiophysicsV. K. Koltov¹, T. A. Evstyukhina², V. G. Korolev², Y. A. Kutlakhmedov³¹Institute of Problems of Chemical Physics, RAS, Chernogolovka, Moscow Region, Russian Federation; ²Petersburg Institute of Nuclear Physics, Gatchina, Leningrad Region, Russian Federation; ³Institute of Cell Biology and Genetic Engineering, NAS, Kiev, Ukraine

Some chemical elements have magnetic and nonmagnetic stable isotopes. This raises the question if the magnetic isotopes can impact living cells, along with the question if they can be of use in medicine. Among three stable isotopes of magnesium, ²⁴Mg, ²⁵Mg and ²⁶Mg with natural abundance 78.7, 10.1 and 11.2%, ²⁵Mg is magnetic (nuclear spin I=5/2) while ²⁴Mg and ²⁶Mg are nonmagnetic (I=0). We studied effects of the magnesium isotopes on post-radiation recovery of yeast cells *S. cerevisiae* irradiated by short-wave UV or X-rays. The enrichment of cells with magnetic ²⁵Mg gives the two-fold increase in the rate constant of the post-radiation recovery compared to the cells enriched with the nonmagnetic isotope. Other model cells, *E. coli*, being enriched with ²⁵Mg, adapt faster to novel growth media by comparison to the cells enriched with nonmagnetic ²⁴Mg or ²⁶Mg. Thus, there are the reasons to believe that ²⁵Mg and, probably, ⁶⁷Zn and some other stable magnetic isotopes, hold much promise in creating novel anti-stress drugs, including radiation protectors, low toxic and suitable for long-term use as nutrition additives.

P-776

Metric analysis for efficient anticancer drug delivery via sonoporation

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In the current study we have performed quantitative evaluation of four main sonoporation factors: 1) MB concentration, 2) US attenuation 3) US scattering and 4) anticancer drug sonotransfer into CHO cells.

MB concentration measurement results and MB cavitation signals, recorded using passive cavitation detection system, were used to quantify sonoporation metrics: 1) MB sonodestruction rate, 2) attenuation rate and 3) inertial cavitation dose. The latter estimates have shown strong, correlation based, interrelations indicating strong physical interdependencies within the processes they represent. All the quantified metrics were successfully used for anticancer drugs, bleomycin and doxorubicin, sonotransfer and cell viability. The results obtained in this study hold promising prospects for eventual development of feedback loop control method for cavitation induced bioeffects.

In addition to this, neither additional bleomycin sonotransfer nor cell viability decrease was observed after complete MB sonodestruction was achieved. This coincides to MB scattered signal spectral root mean square (RMS) and attenuation decrease to background levels. The latter results validate the signal-monitoring based method to determine optimal exposure duration to obtain maximal sonotransfer efficiency.

Posters

– 24. Drug discovery and delivery –

P-777

Characterisation of the radical-SAM enzymes RumMc1/2 by Mössbauer spectroscopy and DFT-calculationsC. S. Müller¹, H. Auerbach¹, S. Chiumento², M. Atta², V. Duarte², C. Basset², J. A. Wolny¹, V. Schünemann¹¹University of Kaiserslautern, Germany; ²CEA - Commissariat à l'énergie atomique et aux énergies alternatives Grenoble, France

Radical-S-adenosylmethionine (radical-SAM) enzymes own a special [4Fe-4S]^{2+/1+}-cluster that activates the substrate SAM generating a radical intermediate that enables the process of chemically challenging reactions [1]. The posttranslational modification of the bacteriocin RumC from the gut microbe *R. gnavus* is conducted via the two radical-SAM enzymes RumMc1 and RumMc2. RumC has an antimicrobial effect on the human pathogen *C. perfringens*. Therefore studies of the biosynthesis and maturation of RumC are important for the development of future antibiotics [2]. Here, a field-dependant Mössbauer spectroscopic study of RumMc1/2 is presented. Information about the cluster structure has been achieved by the development of structural models based on density functional theory (DFT)-calculations.

[1] M. Atta, S. Arragain, M. Fontecave, E. Mulliez, J. F. Hunt, J. D. Luff und F. Forouhar, *Biochimica et Biophysica Acta* 1824 (2012) 1223.

[2] A. Pujol, E. H. Crost, G. Simon, V. Barbe, D. Vallenet, A. Gomez und M. Fons, *FEMS Microbiology Ecology* 78 (2011) 405.

P-779

New lead compounds for Tuberculosis booster by structure-based drug discover with GOLDE. Pohl¹, N. J. Tatum¹, J. C. Coles², N. Willand³, A. R. Baulard³

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The transcriptional regulator EthR from *Mycobacterium tuberculosis*, a member of the TetR family of prokaryotic homodimeric transcriptions factors, controls the expression of the mycobacterial mono-oxygenase EthA. This enzyme is responsible for the bio-activation of the second-line tuberculosis pro-drug ethionamide and since EthR controls EthA expression levels, EthR inhibitors have been shown to boost drug efficacy [1].

Here, we present a comprehensive *in-silico* structure-based screening protocol that led to the identification of a number of novel scaffolds of EthR inhibitors. Biophysical characterization of 85 potential leads and a number of co-crystal structures confirm the binding and inactivation mode of several novel chemical scaffolds.

[1] Blondiaux N, et al. (2017) *Science* 355:1206-1211.

P-778

Venom-derived peptides as therapeutic leads: novel fast-acting insulins

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The venoms of cone snails consist of hundreds of disulfide-rich peptides, some of which target neuroendocrine processes.^[1] We identified a conopeptide targeting energy metabolism, which constitutes a previously unrecognised mechanism of prey sedation.^[2] This insulin, Con-Ins G1, has much greater similarity to fish insulins than to molluscan insulins and has several PTMs (hydroxyproline, γ -carboxyglutamate). Con-Ins G1 is also unusually small, with a shorter B-chain compared to all known vertebrate insulins. This venom insulin elicits hypoglycemic shock in fish, implying that it is used as a weapon for prey capture by a subset of fish-hunting cone snails. We have determined the structure of Con-Ins G1 and modelled its interaction with the insulin receptor.^[3] Comparison with the structure of hIns indicates how the venom peptide compensates for the absence of key receptor-engaging residues in hIns. The potential of Con-Ins G1 to guide the development of truncated, fast-acting human insulin analogues will be discussed.

[1] S. D. Robinson et al., *PLoS One* **2014**, *9*, e87648.

[2] H. Safavi-Hemami et al., *Proc Natl Acad Sci U S A* **2015**, *112*, 1743.

[3] J. G. Menting et al., *Nat. Struct. Mol. Biol.* **2016**, *23*, 916.

P-780

***In silico* studies applied on acetylcholinesterase inhibitors based on natural compounds**

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Severe cognitive and behavioural impairments are induced by the imbalance of neurotransmitters which appears in many psychiatric and neurological disorders. Even if the pharmacological therapies are based on several natural and synthetic chemicals as enzymes and membrane receptor ligands, with still unclear specific mechanisms, the symptoms of Alzheimer disorder (AD) remain severe and the progress of the disease is not halted. Under such circumstances, latest strategies in experimental and *in silico* neuroscience, consider that is critical to identify how already clinically-approved Alzheimer drugs and natural compounds isolated from plants oil are able to improve AD symptomatology. Results of recent studies suggested that acetylcholinesterase and NMDA ligands are able to improve neuroplasticity and improve symptomatology by themselves but many molecular aspects of these processes are still unclear. The data linking natural compounds to improving AD symptomatology is extremely scarce. Here we present the chemical structures-biological activity relationship (SAR) of these molecules revealed by recent experimental and *in silico* studies, offering a new perspective on the molecular mechanism.

Posters

– 24. Drug discovery and delivery –

P-781

Structure-based design of allosteric ecto-5'nucleotidase inhibitors: application in cancer treatmentR. M. Rahimova¹, C. Lionne², L. Chaloin¹¹IRIM – CNRS – UMR9004 – Université de Montpellier, France; ²CBS – CNRS – UMR5048 – INSERM U 1054 – Université de Montpellier, France

Tumor cells use several mechanisms of immunosuppression to facilitate cancer promotion and the development of metastasis. Ecto-5'-nucleotidase (CD73)-generated adenosine is a potent suppressor of the antitumor immune response. CD73 was found to be overexpressed in many cancers with a particular aggressiveness in breast cancers. Thus, new human CD73 inhibitors are required to be used alone or in combination with other anticancer drugs, to struggle cancer progression.

Here, we focused on allosteric inhibitors by combining *in silico* screening and targeted molecular dynamics simulations for the identification of allosteric binding sites. 324 400 compounds were screened by docking in the CD73 target cavity and 33 hits were selected and validated by *in vitro* inhibition assays using recombinant protein or breast cancer cells. The inhibition mode and steady-state kinetics of most potent inhibitors were determined and RR1 was found as non-competitive inhibitor with K_i of 0.15 μ M. Bioinformatics approaches such as pharmacophore models were applied for lead optimization. Co-crystallization trials and biophysical studies of the complex will be performed to provide evidence of its real binding site. New potent and selective CD73 inhibitors will be highly beneficial for anti-cancer therapy.

P-783

Molecular dynamics simulation of a self-assembled complex displaying a GM1 ganglioside clusterY. Tachi^{1,2}, Y. Okamoto¹, H. Okumura^{2,3}¹Department of Physics, School of Science, Nagoya University, Japan; ²Department of Theoretical and Computational Molecular Science, Institute for Molecular Science, Japan; ³Department of Structural Molecular Science, The Graduate University for Advanced Studies, Japan

Recently, a supramolecule that displays a cluster of natural bioactive GM1 ganglioside was reported. The supramolecule is expected not only for a novel analytical tool but also for drug discovery and therapy by adsorbing and removing pathogenic substances *in vivo*. In the previous study, it was reported that the supramolecule recognizes amyloid- β peptide (1-40) but monomeric GM1 sugar does not. This interaction is important for the pathogenic mechanism of Alzheimer's disease.

In this study, we simulated the supramolecule by molecular dynamics method and studied its physical properties. We compared the properties of the GM1 sugar on the supramolecule and that of the monomeric GM1 sugar. As a result, it was found that the dynamics of the GM1 sugar on the supramolecule are more restricted by hydrogen bonding between sugar chains. We will report the details of this analysis at the poster presentation.

P-782

Cellular effects of 3-bromopyruvate, a potential anticancer drug

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Glycolytic inhibitor 3-bromopyruvic acid (3-BP) is a promising anticancer agent since malignant cells are more sensitive to inhibition of glycolysis due to the Warburg effect. 3-BP enters cells via monocarboxylate transporters. We characterized the kinetics of 3-BP transport into erythrocytes and found that flavonoids inhibit this transport, which points to the necessity of avoiding flavonoid supplements during 3-BP therapy. GAPDH is thought to be the main cellular target of 3-BP. However, 3-BP inhibits also other enzymes, including antioxidant ones and induces oxidative stress in the cells. We found that 3-BP inhibits superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione S-transferase in erythrocytes and breast cancer cells, depletes glutathione and induces transient increase in the production of reactive oxygen and nitrogen species. We demonstrated formation of a 3-BP conjugate with glutathione. 3-BP can sensitize cells showing multidrug resistance dependent on overexpression of ABC transporters to cytostatic drugs as ATP depletion inhibits active export of these compounds. We found that 3-BP induces changes in the expression of over 180 genes, including genes coding for control of cell division.

P-784

In silico evaluation of new 5-arylidene(chromenyl)-thiazolidinediones as safer K-Ras inhibitorsR. Tamaian^{1,2}, C. Nastasă³, O. Oniga³, B. Tîpceriuc³¹National Institute for R&D for Cryogenic and Isotopic Technologies, 4th Uzinei street, 240050 Rm. Vâlcea, Romania;²Biotech Corp SRL, 4th Uzinei street, 240050 Rm. Vâlcea, Romania; ³Iuliu Hatieganu University of Medicine and Pharmacy, 41st Victor Babeş street, 400012 Cluj-Napoca, Romania

Mutations of the KRAS gene are the most common genetic abnormalities in cancer. In this study, we evaluated *in silico* with the help of AutoDock Vina a series of previously synthesised 5-arylidene(chromenyl)-thiazolidinedione derivatives (CN01-28) as inhibitors of K-Ras. Additionally, all derivatives were screened with FAF-Drugs3 in order to predict their ADME-Tox properties. Docking results showed a good binding affinity against different K-Ras mutants. Generally, derivatives complied with the threshold values of drug-likeness filters; CN01-03 and CN07-11 had one violation of Lipinski's rule of five in terms of LogP. The predictions indicated a good bioavailability. The screening for PAINS was negative and all derivatives were qualified as phospholipidosis non-inducers. CN16-25 were rejected as non-peptidic inhibitors of protein-protein interactions, moreover CN21-25 have a high-risk moiety (thiazolidinedione). The most promising derivatives as safer K-Ras inhibitors are CN04-06, CN12-15 and CN26-28.

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Posters

– 24. Drug discovery and delivery –

P-785

New anti DNA topoisomerase naphthalenedione leads

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Human Type II DNA Topoisomerase is one of the oldest onco-targets, however development of safer inhibitors is still a challenge. In this paper, we screened a combinatorial library with naphthalenedione derivatives for development of new TOP2A inhibitors. We use SmiLib v2.0 to build the combinatorial library, by virtual reactions using as scaffold molecule a naphthalenedione moiety and a building blocks set with structural motifs generally found in antimetabolite and antineoplastic drugs. The entire library was virtually screened with FAF-Drugs3 for prediction of their ADME-Tox properties. AutoDock Vina was used to predict the binding mode and affinity (kcal/mol) of the most promising lead-like naphthalenedione derivatives. At the end of virtual screening a very short list of leads was identified to be undergone for further modifications in order to be drug-like enough for organic synthesis and *in vitro* testing.

Acknowledgments: this research was financed by UEFISCDI, Romania, on the Contract no. 210/2014, Project PN-II-PT-PCCA-2013-4-2075 and 34N/2016 NUCLEU Program, Project PN 16 36 04 03.

P-787

Thermal stability, storage, release and delivery of insulin – A protection through sol-gel tailored silica

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Stability of biological substances based on proteins, including vaccines and drugs, is critically linked to its thermal environment. Their storage and distribution therefore relies on a “cold chain”. This is costly and not always effective in medical applications. Diabetes is predicted to be the 7th leading cause of death. According to WHO, access to insulin is still beyond the reach of millions diabetes. The need for a cold chain during transport and storage has caused poor insulin availability and affordability. We have published a protocol of “ensilication”, enclosing protein in a deposited silica “cage” to prevent the protein from the denaturing process.¹ The investigation was continued onto the mechanism of the ensilication using SAXS and DLS, and an artificial digestion system has also been tested for oral delivery. This innovative method has been applied to insulin, which is also served as a model of negative charged proteins. The research has a profound impact on our ability to store temperature sensitive matter and remove our constant dependence on cold-chain, enabling insulin and other biological materials to be taken anywhere in the world without refrigeration.

P-786

NMR Structural Study of a PASylated Protein

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PEGylation of biopharmaceuticals is routinely used to increase their hydrodynamic volume and circulation half-life. However, PEGylation can be costly due to additional purification steps, and can impede biological activity. Fusion proteins with a conformationally disordered polypeptide, exhibiting PEG-like properties, are being developed as an alternative to PEGylation. Schlapschly *et al.* recently described PASylation, in which the fusion consists of Pro, Ala, and Ser (PAS) residues¹. We have used a model protein RSL^{2,3} with a C-terminal PAS motif to study the effect of PASylation on the protein structure. ¹H-¹⁵N HSQC spectra reveal resonances corresponding to the PAS motif at around 8.5 ppm, indicative of unfolded protein and consistent with the disordered nature. An analysis of chemical shifts of RSL-PAS revealed that the RSL resonances were consistent with the native molecule² with minor changes at the C-terminus and adjacent residues. In-cell NMR data of RSL-PAS will also be presented.

1. M. Schlapschly *et al.*, *PEDS*, **2013**, *26*, 4892. P. Antonik *et al.*, *Biochemistry*, **2016**, *55*, 11953. P. Antonik *et al.*, *Biomacromolecules*, **2016**, *17*, 2719

P-788

A novel long-circulating liposomal formulation of docetaxel ensuring higher stability

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Docetaxel (DTX) is commonly used as chemotherapeutic in the treatment of many types of cancer. The disadvantages of using DTX in therapy are its low solubility in water, as well as the toxicity and poor pharmacokinetics of existing formulations. Using lipid carriers, such as liposomes, would seem an attractive approach in overcoming these problems. However, using liposomal formulations tested so far is limited by the low level of DTX molecules that may be encapsulated within liposome membranes and which are mostly accompanied by drug crystallization in the liposomal suspensions. In an attempt to stabilise the incorporated drug, we used pegylated HSPC liposomal formulations containing a novel synthetic 3-*n*-pentadecylphenol derivative, named KW101/III. Our data showed a remarkably enhanced stability of the DTX liposomes, characterized by the inhibition of DTX crystallization over 40 days, in comparison to 2 days in the formulation without the KW101/III, alongside a higher drug loading of 4 mol%, compared to the 2.8 mol% loading achieved in the original liposomal formulations. Moreover, this formulation showed remarkably improved pharmacokinetics in blood, in comparison to other control formulation composed of ePC, which is commonly used as a liposomal carrier for DTX.

Posters**– 25. Motility and migration –****P-789****Ring and bundle formation in confined cross-linked actin filament networks**

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Actin filaments form a range of networks that the results of competition between shape and size of the confinement, actin concentration, and type of the cross-linker. We performed numerical simulations to investigate the effect of spherical confinement and cross-linking. We used Brownian dynamics to simulate semi-flexible actin filaments polymerizing in a confining sphere. We investigate the effect of implicit cross-linking in the equilibrium configuration and present a phase diagram based on the length and concentration of filaments, the range and strength of crosslinking interaction, and sphere radius. We quantify these systems by their radial distribution, planar order parameter and ring formation probability. The simulations reproduce ring structures that form between unbundled and collapsed phases. We also developed simulations with explicit cross-linking between filament beads separated by a distance equal to the actin double-helical half-pitch. This method allows us to model the effect of stiff parallel cross-linkers such as fascin and compare to the case of flexible cross-linkers such as α -actinin. We explore the effect of the fascin concentration in persistence length and thickness of the bundles and compare the results with implicit cross-linking case.

P-791 (O-148)**Instabilities of competing tissues with mechanically-cued proliferation**

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The Francis Crick Institute, UK

Inspired by tissue replacement processes such as in the developing abdominal epidermis of *Drosophila*, we model a propagating interface between competing epithelial tissues in which cell division and death are mechanically cued. We derive the steady propagation in 1D and show how this can be driven either by an imbalance in “homeostatic pressure”, or by active, directed motility forces present in one or both tissues. Then, we determine whether the interface is stable against stochastic fluctuations of its contour. We find that a number of destabilising effects are possible. One of these involves the substrate friction and is reminiscent of the Saffman-Taylor or “viscous fingering” instability, which occurs in non-living systems and is well-known in oilfield engineering. The interface can also become unstable on longer length scales via an effective viscosity associated with cell division and death. Active motility forces play a complex role, through which even a stationary interface may be unstable or stable depending on the direction of motility forces in each tissue. Our results provide insight into instabilities arising purely from the effective material parameters of mechanosensitive tissues.

P-790 (O-147)**4D fast quantitative imaging of vascular invasion: the role of cell-matrix mechanical interaction**C. Steuwe^{1,2}, M.-M. Vaeyens², A. Jorge-Peñas², M. Roeflaers¹, H. van Oosterwyck^{2,3}¹Centre for surface chemistry and catalysis, KU Leuven, Belgium; ²Biomechanics Section, KU Leuven, Belgium; ³Prometheus-Mechanobiology, KU Leuven, Belgium

Tissue engineering aims at recapitulating development, growth and regeneration of tissues and organs. It is therefore vital to assess multicellular processes in a fast, quantitative and non-invasive way. Here we present an approach to study sprouting angiogenesis in 3D with high temporal resolution and under chemically defined culture conditions. Multimodal and fast lightsheet microscopy was used to acquire stacks of markers during the live sprouting of human umbilical vein endothelial cells and after their chemically induced relaxation. The calculation of the matrix deformations was formulated as a 3D non-rigid image registration process and is based on data either from beads or from fibers. Using this technique we deduct highly temporally resolved mappings of traction-induced matrix deformations of a 3D collagen matrix around angiogenic sprouts. It allows to gain quantitative understanding on rapid mechanisms that govern multi- and intercellular dynamics under various conditions.

P-792**Swimming in extreme environments**

R. C. Findlay, P. B. Walrad, L. G. Wilson

University of York, UK

The ability to swim or pump fluid is critical to the survival of many cells, including many that are of biomedical relevance (i.e. pathogenic microorganisms). These pathogens may have to survive in multiple hosts: for example, insect-borne pathogens have several distinct and widely differing conditions that they must navigate, avoiding or exploiting their hosts' immune systems in order to propagate and spread. We have used holographic microscopy to explore the physics of swimming in an important cellular pathogen – the eukaryotic parasite *Leishmania mexicana*. This eukaryote radically remodels its anatomy during its life cycle, as it progresses from the insect-borne to the vertebrate-borne phases. We have physically characterized this species' swimming apparatus (a eukaryotic flagellum) at key points in the life cycle, finding intriguing data on the ‘optimal’ configuration at each stage.

Posters

– 25. Motility and migration –

P-793

Quantitative, time-resolved, 3D optical analysis of bacterial co-cultureA. L. Hook¹, J. L. Flewellen², N. Russell¹, P. Williams¹, M. R. Alexander¹¹University of Nottingham, UK; ²The Francis Crick Institute, UK

Bacterial attachment and subsequent biofilm formation on medical devices remains a major healthcare challenge, leading to persistent device-associated infections with a concomitant increase in patient morbidity and mortality. These systems result from complex interactions between heterogeneous populations of cells including interspecies interactions. Quantitative, time-resolved, 3D analysis at the single cell level is necessary to develop a deeper understanding of dynamic infection processes¹. As well as bacterial motility and behaviour within the bulk, bacterial surface interactions are a key component of the biofilm life-cycle. To study this environment effectively we have developed a multimode microscope with digital holography, total internal reflection, differential interference contrast, and remote-image z-stacking capabilities. We present results from the use of this system to observe the interaction of motile *Pseudomonas aeruginosa* with surface-adhered *Staphylococcus epidermidis* to robustly assess their behaviour, altered as a result of the co-culture environment.

1.Kemper, B. et al., *Journal of Biophotonics* **2013**, 6 (3), 260-266.

P-795

A model for the nut-and-bolt mechanism of phage migration along bacterial flagellaP. Katsamba, E. Lauga
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Bacteriophage viruses have the striking appearance of microscopic spaceships. One of the most abundant entities in our planet, they crowd fluid environments in anticipation of a random encounter with bacteria, and use a remarkable nanometre-size machinery for infection: fibres that recognise and attach to specific receptors on their victim's surface and a hollow tube through which their genetic material is ejected inside the host cell cytoplasm for replication. Flagellotropic phages first attach to the flagella of bacteria and find a way to reach the cell body for infection since they lack the ability to move independently. The means by which they move up the flagellum has intrigued the scientific community for over 30 years. In 1973 Berg and Anderson proposed the nut-and-bolt mechanism and 26 years later, Berg's group provided supporting evidence for it. Just like a nut being rotated will move along a bolt, under this scenario the phage wraps itself around a flagellum possessing helical grooves (due to the helical rows of flagellin molecules) and exploits the rotation of the flagellum in order to passively travel along it. In this work, we provide a first-principle theoretical model for this nut-and-bolt mechanism and show that it is able to predict experiment observations.

P-794

Measurement of the stall torque generated by the bacterial flagellar motorT. Kasai¹, Y. Sowa^{1,2}¹Research center for Micro-Nano Tech., Hosei Univ, Japan;²Dept. Frontier Biosci., Hosei Univ., Japan

The bacterial flagellar motor is a rotary molecular machine which is powered by the ion flux across the cell membrane. The motor consists of a rotor of 50 nm in diameter surrounded by up to ~11 stator units. Multiple stator units can interact simultaneously with the rotor to generate torque in a motor. The stator units dynamically exchange between the motor and a pool in the cell membrane, and the incorporation of stator units in the motor depends on the mechanical load to the motor. Therefore, the measurement of motor torque under controlled load conditions is important for understanding energy conversion mechanism of the motor. In this study, we measured the stall torque of the motor by combining optical tweezers and nanometry. The rotating tethered cell was stalled by a trapped polystyrene bead and the generated torque was estimated to be about ~2000 pN nm from the displacement of bead position. The torque at stall was larger than the torque generated by a spinning motor at low speed. This large stall torque could be caused by either or both of increase of incorporated units in a motor and torque generation by a single stator unit.

P-796

Osmotaxis in *E. Coli* through changes in motor speedJ. Rosko¹, V. A. Martinez², W. C. K. Poon², T. Pilizota¹¹School of Biological Sciences, University of Edinburgh, UK;²School of Physics and Astronomy, University of Edinburgh, UK

Bacterial motility, and in particular repulsion or attraction towards specific chemicals has been a subject of investigation for over a 100 years, resulting in detailed understanding of bacterial chemotaxis and the corresponding sensory network in many bacterial species. *Escherichia coli* can be both a commensal bacterium and a pathogen, and ongoing work aims to identify niches where motility and taxis could be important when invading a host.

Most of our current understanding of taxis comes from experiments with low levels of chemotactically active ligands. However, inactive chemical species have been shown to mechanically activate the chemosensory network at concentrations which produce significant changes in external osmotic pressure. To understand how these nonspecific physical signals interact with chemical signals, and influence motility, we look into the tactic response of *E. coli* when exposed to changes in osmolarity that are similar to those found in the human gastrointestinal tract.

Combining a populational swimming assay with a single cell approach that directly probes the output of the chemosensory network, we observe long term increases in swimming speed and reorientation frequency in response to step-like upshifts in osmolarity. We discuss how changes in these parameters can lead to bacterial accumulation.

Posters**– 25. Motility and migration –****P-797****Polarization dynamics of single cells and small groups of cells on micropatterns**S. A. Schaffer¹, A. Goychuk², F. Zhou¹, C. Schreiber¹, E. Frey², J. O. Rädler¹¹Faculty of Physics, Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 Munich, Germany;²Arnold Sommerfeld Center for Theoretical Physics, Ludwig-Maximilians-Universität, Theresienstr. 37, 80333 Munich, Germany

The GTPases Rac1 and RhoA are key regulators of actin dynamics in cell migration. At the front of the polarized cell, Rac1 drives actin branching, whereas RhoA controls actomyosin contractility at the back. Understanding how key regulators accomplish cell polarity and how polarity is communicated to neighboring cells is of fundamental importance to connect single cell- to collective cell-migration.

Micropatterns allow for controlled confinement of cell motion and hence systematic investigation of external cues and internal fields. We studied migration of breast cancer cells on short lanes, ring-shaped lanes and Y-shaped lanes using Life-act labeling and a marker for Rac1. To investigate the role of focal adhesions and the effect of cell to cell coupling, we designed specific cell collision or contact patterns.

The dynamic actin patterns are compared to simulations with a Cellular Potts Model (CPM) that includes internal dynamics of cytoskeleton components. In the long run, we want to calibrate the CPM for different cell lines, which will then allow us to specifically modify certain parameters without the experimentally omnipresent cross-talk of regulatory molecules.

P-798**Characterizing single cell migration and transitions to different substrate coatings**C. Schreiber¹, F. J. Segerer¹, E. Wagner², A. Roidl², J. O. Rädler¹¹Faculty of Physics, Ludwigs-Maximilians-Universität, Munich, Germany; ²Department of Pharmacy, Ludwigs-Maximilians-Universität, Munich, Germany

Deviations from the typical migratory behavior of a cell can often be an indicator for pathological conditions. Hence, finding suitable metrics to characterize cell motility is of fundamental importance in pathology as well as in drug screening. Furthermore, migrating cancer cells are faced to different extra cellular matrix proteins that can influence the cell migration behavior. Here, we use micro patterned stripes or rings to constrict cell migration to 1D and create defined junctions where the substrate coating changes. For homogeneous surface coating we find bimodal behavior with states of directional migration (run state) and reorientation (rest state). We extract characteristic persistence times which, in combination with the velocity of cells in the run state, provide a set of parameters quantifying cell motion. At transitions to cell repellent PEGylated areas cells reverse or sometimes traverse if the PEGylated area is small enough. For transitions of different ECM proteins like fibronectin and collagen IV cell migration behavior and focal adhesion dynamics are studied. Together this results in a fingerprint-like set of parameters characterizing cell migration that can be used to distinguish cell lines as well as to quantify the effects of motility affecting drugs.

Schreiber, C et al. Sci. Rep. 6, 26858 (2016)

Posters

– 26. Applications in biomedical and materials science –

P-799

Mechanics of ageing collagen from molecule to tissueA. Gautieri^{1,2}, F. Passini¹, M. Guizar-Sicairos³, A. Redaelli², J. G. Snedeker¹¹Institute for Biomechanics, ETH Zurich, Switzerland;²Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy; ³Paul Scherrer Institute, 5232 Villigen PSIVilligen, Switzerland

Concurrent with a progressive loss of regenerative capacity, connective tissue aging is characterized by a progressive accumulation of Advanced Glycation End-products (AGEs). Although AGEs are associated with a wide range of clinical disorders, the mechanisms by which AGEs contribute to connective tissue disease in aging and diabetes are still poorly understood. The present study harnesses advanced multi-scale imaging techniques to characterize a widely employed *in vitro* model of ribose induced collagen aging and further benchmarks these data against experiments on native human tissues from donors of different age. These efforts yield unprecedented insight into the mechanical changes in collagen tissues across hierarchical scales from molecular, to fiber, to tissue-levels. We observed a linear increase in molecular spacing (from 1.45 nm to 1.5 nm) and a decrease in the D-period length (from 67.5 nm to 67.1 nm) in aged tissues, both using the ribose model of *in vitro* glycation and in native human probes. Multiscale mechanical analysis of *in vitro* glycated tendons strongly suggests that AGEs reduce tissue viscoelasticity by severely limiting fiber-fiber and fibril-fibril sliding.

P-801 (O-156)

Micro-structured compartment models for synthetic biologyH. Eto¹, M. Heymann¹, N. Soga², H. Noji², P. Schwill¹¹Max-Planck-Institute of Biochemistry, Martinsried, Germany; ²Department of Applied Chemistry, University of Tokyo, Japan

Vesicle bilayer systems have been extensively employed to investigate protein-membrane interactions. When investigating peripheral membrane proteins, it often suffices to replace the bilayer by monolayer and work in water-in-oil droplets, which are more flexible and versatile in preparation. Microstructured wells coated by membrane have been used for this purpose, which, however, often pose the challenge of proper sealing. We investigate compartmentalised droplet-bilayer interfaces that encapsulate femto-litre droplet volumes under a free-standing bilayer. Based on fluoro-polymer chemistry, these microdevices can be patterned into various geometries and can facilitate high-throughput assays to analyse membrane-intercalating or interfacing proteins. We discuss the reconstitution of spatially self-organizing proteins in these isolated-volume microcompartments.

P-800 (O-155)

Atomic force microscopy as a tool to evaluate the risk of cardiovascular diseases in patientsA. F. Guedes¹, F. A. Carvalho¹, I. Malho¹, N. Lousada², L. Sargento², N. C. Santos¹¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; ²Unidade de Insuficiência Cardíaca, Departamento de Cardiologia, Hospital Pulido Valente, Centro Hospitalar Lisboa Norte, Lisbon, Portugal

The availability of biomarkers to evaluate the risk of cardiovascular diseases is limited. High fibrinogen levels have been identified as a relevant cardiovascular risk factor but the biological mechanisms remain unclear. Increased aggregation of erythrocytes (red blood cells) has been linked to high plasma fibrinogen concentration. Here, we show using atomic force microscopy, that the interaction between fibrinogen and erythrocytes is modified in chronic heart failure patients. Ischemic patients showed increased fibrinogen-erythrocyte binding forces compared with non-ischemic patients. Cell stiffness in both patient groups was also altered. A 12-month follow-up shows that patients with higher fibrinogen-erythrocyte binding forces initially were subsequently hospitalized more frequently. Our results show that atomic force microscopy can be a promising tool to identify patients with increased risk for cardiovascular diseases. [Guedes *et al.* (2016) *Nature Nanotechnol.*, doi:10.1038/nnano.2016].

P-802 (O-157)

Cellular sensing platform for biomedical applicationsL. Stanica^{1,2}, M. Gheorghiu¹, E. Gheorghiu^{1,2}¹International Centre of Biodynamics, Intr Portocalelor, Nr 1B, 060101, Bucharest, Romania; ²Faculty of Biology, University of Bucharest, Splaiul Independentei, Nr 91-95, 050095, Bucharest, Romania

High-sensitive methods that provide noninvasive real-time detection of cellular responses to pharmacological agents and biochemical compounds with therapeutic potential are needed in biomedical research. To this end, we advance a cellular platform integrating electrical impedance spectroscopy (EIS) and a model cell line (human colon cancer HT-29 cells) for investigating the effectiveness of emergent Carbonic Anhydrase Inhibitors (CAIs) (sulfonamides and sulfocoumarins) as anticancer drugs.

EIS enabled label-free, noninvasive real-time evaluation of the effect of CAIs on HT-29 cell monolayers subjected to hypoxic conditions. The results reveal specific alterations occurring within the cell layer, especially at the cell-surface level induced by the application of CAIs and hypoxic conditions. Factors like changes in cell-substrate adherence, cell number and cell-cell interactions influence the measured signal thus allowing discriminating the inhibitory capacity of the compounds directed against CA IX, a tumor specific metalloenzyme regulating extra- and intercellular pH towards tumour survival growth and metastasis. Our results indicate a powerful biosensing approach for the evaluation of CAIs potency, effective for anticancer pharmacological agents screening and design.

Posters

– 26. Applications in biomedical and materials science –

P-803

Unveiling the role of the mutation F508del in cystic fibrosis

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Cystic fibrosis is a genetic disease that causes the accumulation of mucus in epithelia, mainly affecting the airways of the lungs. These secretions not only cause obstruction, but also inflammation and infections by *Pseudomonas* and *Staphylococcus* organisms. The mutated gene in this disease encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is a chloride channel. Although there are over 1500 mutations related to cystic fibrosis, the most common is the deletion of phenylalanine 508 (F508del). F508del causes the misfolding of the CFTR polypeptide leading to its degradation in the ER. Nevertheless, a small amount of defective channels is still able to reach the cell membrane, but display an impaired function leading to minimal chloride transport.

The CFTR channel belongs to the class of ABC transporters. These proteins have been extensively studied using computational methods in our lab in order to clarify their mechanism and mode of action.

In the present work, we derived a new model for the NBD1-NBD2 association of human CFTR based on existing data using comparative modelling techniques, and performed molecular dynamics simulations of the CFTR protein in both mutant and wild-type forms with the goal of studying the conformational consequences of ATP hydrolysis in the mutant and wild-type forms.

P-805

Iron doped calcium phosphate biomaterials for tissue engineering

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Calcium phosphate minerals, due to their chemical similarity with the natural mineral of bone have been widely used for tissue engineering applications. Recently, we demonstrated that doping these minerals with iron improves their laser absorption and mechanical properties and made them an attractive option for manufacturing bone scaffolds using selective laser sintering SLS. The present work aims to further investigate the suitability of the Fe-CaP materials for hard tissue scaffolds and their interaction with cells. Powders with four different concentrations of iron (0%, 5%, 10% and 20%) were synthesised and tested for laser absorption, mechanical properties, and cytotoxicity. We observed that the biocompatibility of these minerals is not affected by the iron concentration while the Fe-doped materials demonstrate better mechanical properties. Finally it was found that the absorbed laser energy increases significantly with the increase of iron concentration.

P-804

Mössbauer spectroscopy and magnetization study of normal and pathological liver and spleen tissues

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Nanosized iron cores in the iron storage proteins ferritin and hemosiderin deposited in various tissues demonstrate different structures and iron contents depending on various factors. For instance, these factors are: (a) type of the tissue, (b) feeding and environmental conditions, (c) pathological processes, etc. Mössbauer spectroscopy and magnetization measurements are very useful techniques for comparison between ferritin-like molecules in healthy and in patients tissues. Therefore, we have studied several human liver and spleen tissues from healthy persons and patients with hematological malignancies using Mössbauer spectroscopy and magnetization measurements in order to obtain information about peculiarities of the iron cores structure and composition. These data may be helpful for analysis the diseases in their molecular level. The results obtained showed differences in the iron content and in the ferritin-like iron core structures as well as in tiny amount of magnetite and paramagnetic components in the healthy and pathological tissues. This work was supported by the Ministry of Education and Science of the Russian Federation (Project # 1959).

P-806

Use of femtosecond lasers for exogenous mineralization of dental enamel

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Since dental enamel is an acellular tissue, any defect or damage is permanent due to the lack of natural remineralisation. To deal with the need of an effective restorative procedure we propose a radically new approach that utilises femtosecond pulsed lasers and Fe²⁺, Fe³⁺ doped calcium phosphate materials (e.g. hydroxyapatite). A layer of the iron rich material is applied on to the surface of enamel. During femtosecond laser irradiation (1040nm wavelength, 1GHz repetition rate and 0.4W av. power), the Fe₂O₃ nanoparticles produce heat (due to photothermal effect) and trigger the sintering and densification of the calcium phosphate crystals which eventually are bonded with natural enamel. The existence of the Fe oxide nanoparticles ensures a localised temperature rise and minimal thermal damage to natural tissue. The hardness of the new layer is comparable with that of dental enamel while after three weeks of brushing trials we found that the wear rate of the new material is much slower than that of natural enamel.

Posters

– 26. Applications in biomedical and materials science –

P-807

The features of interactions of porphyrins with A-DNA

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The features of interactions of porphyrins with A-DNA.

There are a number of works were devoted for B-DNA-porphyrin complexes, but studies with the A-form of DNA are almost absent. Thus, studies of the features of interactions of A-DNA with porphyrins very relevant. In the present study we investigated binding properties and the thermodynamic parameters of complexes of water-soluble cationic meso-tetra-(4N-oxyethylpyridyl) porphyrin H₂TOEPyP4 and their Cu-, Co- derivatives with A-DNA. All studies were performed at ionic strength $[Na^+] = 10^{-3}$ M, pH 7.0. The peculiarities of binding the porphyrins with A-DNA have been studied by UV-vis spectrophotometry and circular dichroism (CD) methods. The induced CD spectra show, that the investigate porphyrins interact with A-DNA preferentially through external groove binding mode. The binding parameters were determined based from absorbance spectra.

The free energies, enthalpies and entropies have been evaluated from data on temperature dependence of binding constants. It was shown that the interactions of all porphyrins with A-DNA are accompanied by positive changes of enthalpies and entropies. The increase of entropy shows that the nature of interaction of porphyrins with A-DNA is predominately hydrophobic.

P-809

Biomechanical studies with ultrasound in cell biology

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During tissue development and cancer progression, cells undergo mechanical changes and respond differently to physical cues from their environment. Usually measuring physical properties of biological samples relies on direct physical contact with the sample. We propose ultrasound as a versatile tool for imaging and also exerting forces on cells that does not require direct contact.

Using a 40 MHz microultrasound transducer, we compared radiofrequency signals with optical images of 3D tissue structures. This revealed that the size of spherical structures correlates with the ultrasound RF signal. Ultrasound overestimates the size of hollow cysts by 33%, while no such offset was observed in solid spheroids. Early results also suggest that microultrasound can reveal mechanical properties.

At lower frequencies (~ 4 MHz) and higher intensities, ultrasound can compress cell layers via acoustic radiation pressure and without the need to directly contact the sample. This allowed investigating how cells react to external mechanical forces.

Developing this approach further will permit comparison of mechanical properties of tissue at different stages of cancer progression and increase our understanding of how mechanical forces direct tissue behaviour.

P-808

Photophysical characterization and fluorescence imaging of *Helicobacter pylori* endogenous porphyrins

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Helicobacter pylori (Hp) is among the most common infective agents in humans and is responsible for several gastric infections; moreover, its antibiotic resistance is raising new challenges. Antimicrobial photodynamic therapy (PDT) is a promising technique based on the use of non-toxic photosensitizers that, under the effect of a selected visible light, can generate cytotoxic reactive oxygen species; PDT is particularly effective when the target microorganism presents endogenous photosensitizers.

It has been shown that the photosensitizers protoporphyrin IX and coproporphyrin I are endogenously produced in Hp, making it a suitable target for PDT. Aiming at the production of an ingestible LED-based robotic pill for intragastric PDT able to perform *in situ* irradiation without the use of endoscopic devices, the effectiveness of Hp photokilling in models has to be determined. We studied the distribution of porphyrins within bacteria suspensions and biofilms and adapted a protocol for porphyrins extraction from bacteria to characterize the spectroscopic features of the extracts.

Project: “CapsuLight - Design of an ingestible robotic pill based on LED sources for the treatment of gastrointestinal disorders” (CUP B52I14005760002) financed by Regione Toscana Bando FAS Salute 2014 (Italy).

P-810

The effects of pore-forming agent nystatin on biological membranes

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The responses of Chinese hamster ovary epithelial cells (CHO cells), giant unilamellar vesicles (GUVs) and giant plasma-membrane vesicles (GPMVs), caused by nystatin, were studied using phase-contrast, brightfield and fluorescence microscopy. In experiments with CHO cells different phenomena, i.e., the detachment of cells, the formation of blebs, the occurrence of “cell-vesicles” and cell ruptures, were observed. These phenomena were compared to similar phenomena discovered in GUVs, i.e., membrane protrusions, transient tension pores, slow vesicle ruptures and explosions. The observed tension-pore behavior in cells and vesicles was interpreted with a theoretical model based on the osmotic effects induced by the occurrence of size-selective nystatin pores. The increase of the cellular volume was predicted and correlated with the observed phenomena. In experiments with GPMVs the observed bead-like protrusions are significantly shorter than those observed in GUVs at the same nystatin concentrations. In both cases the appearance of protrusions can be explained considering the intercalation of nystatin into the outer membrane monolayer since this increases the corresponding surface area.

Posters

– 26. Applications in biomedical and materials science –

P-811

Application of titanium oxide carbide hybrid as a new biomaterial

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A new compact hybrid material: titanium oxide carbide has been synthesized. TiO₂ nanotube layers were converted using a robust carbo-thermal reduction treatment into an oxy carbide compound. Titanium with its native oxide layer belongs to the most successful medical implant materials. The presence of carbon ensures electrical conductivity. The new material reflects the IR and visible light. This unique combination of physiochemical properties in this new hybrid material makes it attractive for *in situ* spectroelectrochemical investigations of assemblies of biomolecules.

The adsorption of proteins on solid surfaces from their aqueous solutions has an important biological and technological meaning. The adsorption process occurs immediately when a protein solution contacts a solid surface and it may have either beneficial or detrimental effects. The adsorption process of collagen type I from rat tail, bovine serum albumin and fibronectin from bovine plasma on the surface of titanium oxide carbide was studied using PM IRRAS. Amide I mode, sensitive to the secondary structure of a protein, was analyzed. Changes in intensities and positions of deconvoluted amide I mode reflect both changes of the secondary structure as well as the orientation of the protein in the adsorbed film.

P-813

Introduction of differential scanning calorimetry (DSC) in the diagnostics of joint capsule damagesG. Bücs¹, L. G. Nöt¹, Á. Dandé¹, N. Wiegand¹, D. M. Lőrinczy²

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Because of the growing number of total hip replacements (THR), new developments are introduced in the operational technique. The problem is whether the joint capsule during THR should be resected or preserved, based on the primary conditions. The DSC is a useful tool in the evaluation of septic/non-septic arthritis. Our purpose was to evaluate whether there is a specific pattern in the thermal characteristics of joint capsule samples, collected from patients with arthritis, avascular femoral head necrosis (AVN) and femoral neck fractures (Fx). The capsule samples were harvested during THRs and considered to be a waste material. The thermal parameters (denaturation temperature T_m , calorimetric enthalpy change, ΔH_{cal}) were monitored by Micro DSC-II calorimeter between 37–90°C with 0.3 Kmin⁻¹ heating rate. The denaturation scans of different joint capsule samples have clearly demonstrated specific changes in case of different samples. The significant differences in the thermal characteristics of arthritic and AVN samples compared to the FXs (T_m (°C) arthritis: 64.7 and AVN: 65.3 vs. FX: 63, $p < .05$; ΔH_{cal} (J/g) arthritis: 9.4 and AVN: 8.9 vs. FX: 5.7, $p < .05$) could be explained by the degenerative or inflammatory conditions.

P-812

Vertical arrays of nanostructures: new nanotool for extra and intracellular studies

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Vertical arrays of nanostructures (NSs) are emerging as promising platforms for probing and manipulating live mammalian cells. The broad range of applications for single cell studies by electrophysiology and fluorescence microscopy requires different types of interfaces, but cell interface with NS arrays is not yet fully controlled and understood.

Here we present a model enabling to predict the influence of nanotopography on cell / NS interface and thereby design the NS arrays adapted to each kind of cellular applications. We furthermore show how we can use arrays of NS to insert non cell permeable compounds such as antibodies and protein sensors in live cells, while this usually requires cell permeation and single point measurements on dead cells.

This strategy provides arrays of NS unique features for live cell activity studies, not possible with classical methods. Being furthermore compatible with any cell type, including nongenetically modified cells, NS arrays are expected to enable novel biological information in the near future.

P-814

How PEGylation of Phosphoglycerate Kinase may change its structure and biological functionality?

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The covalent conjugation of PEG to proteins is an established technique for enhancing the pharmacokinetic properties of protein therapeutics. The properties of PEG such as good water solubility, lack of toxicity and low immunogenicity can be useful in drug design as it can increase drug solubility, increase circulating time or reduce immune response. The process of PEGylation can be applied to enzymes and may be used in Enzyme Replacement Therapy (ERT), which is the medical method of treatment for patients with enzyme deficiency. However, the PEG attachment to protein can cause decrease of enzymatic activity. The PEG attached to protein may limit access of substrates to active site. In our present study we focus on Phosphoglycerate kinase (PGK) to observe the influence of PEGylation on structure, dynamics and activity of enzyme. Our goal is to test whether PEGylation changes the conformation and/or interrupts the cleft closing of PGK induced by ligands. A combination of SAXS, Circular dichroism, Enzymatic assay may help us to understand the real influence of mPEG on structure and function of PGK. Additional Neutron Spin Echo Spectroscopy is used to directly access the domain motion of PGK and the influence of PEGylation, which is relevant for PGK activity. This may help to understand how to increase the efficiency of PEGylated enzymes in ERT.

Posters

– 26. Applications in biomedical and materials science –

P-815

Enzyme biosensor development and evaluation as sensitive tool for biomedical applicationsM. David^{1,2}, M. Florescu²¹Faculty of Physics, University of Bucharest, Magurele, Romania; ²Faculty of Medicine, Transilvania University of Brasov, Brasov, Romania

Enzyme immobilization methods play an important role for biosensor performance, protecting enzyme bioactivity and it's successfully integration onto the electrode surface. In this work, we focused on two entrapment methods, using polymers and nanomaterials in order to improve biosensor performance.

A tyrosinase-based biosensor was developed for dopamine (DA) detection. Gold electrodes were previously modified with cobalt (II)-porphyrin polymeric film, upon which tyrosinase was cross-linked. Layer-by-Layer method was also used to modify gold electrodes surface for glucose detection. Glucose oxidase and a variety of carbon nanomaterials were dispersed in the positively-charged polymer chitosan, together with the negatively charged polymer poly(styrene sulfonate), PSS⁻.

Electrochemistry and surface plasmon resonance were used for biosensors evaluation. By optimizing methods parameters, selective detection of DA and glucose was facilitated in both situations with very good sensitivity and detection limit in nanomolar range. Biosensors were tested in both cases in the presence of real samples and obtaining good results.

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P-817

Application of natural surfactant protein fusions to direct cell adhesion on hydrophobic substrates

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A common approach to tissue engineering is to seed cells onto 2- or 3-dimensional physical scaffolds that provide shape, mechanical and nanotopographical cues that direct the attachment and development of the cells. Many of the most commonly used substrates are hydrophobic polymers that lack intrinsic cell adhesive properties thus necessitating harsh and time-consuming physicochemical treatments.

Natural surfactant proteins such as ranaspumin-2 (Rsn-2) adhere to hydrophobic surfaces, and Rsn-2 alone will render hydrophobic substrates cell compatible. We have exploited this property to generate fusion proteins in which Rsn-2 tethers peptide sequences derived from integrin binding proteins to hydrophobic substrates providing enhanced cell adhesion and developmental cues.

P-816

Novel tyrosinase-based biosensor for real sample dopamineM. David^{1,2}, M. Florescu²¹Faculty of Physics, University of Bucharest, Magurele, Romania; ²Faculty of Medicine, Transilvania University of Brasov, Brasov, Romania

Detection of dopamine (DA), an important neurotransmitter, is linked to a large variety of medical conditions, such as Parkinson or attention deficit hyperactivity disorder, which lack a simple, reliable diagnosing tool for early detection.

A novel tyrosinase-based biosensor was developed for rapid, sensitive and selective detection of DA in the presence of main interferent, ascorbic acid (AA). Electrochemistry and spectroscopy were used for biosensor characterization and selective dopamine detection. Gold electrodes were previously modified with cobalt (II)-porphyrin film, for increased selectivity detection. By optimizing parameters, a separation between the oxidation peak potentials for AA and DA was obtained. The tyrosinase-based biosensor reached a sensitivity of $1.83 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$ and a detection limit of $0.59 \mu\text{M}$. Due to its good reproducibility and stability, the biosensor was tested in the presence of real dopamine sample, with satisfactory results in terms of recovery and relative standard deviation values. These results determine the biosensors applicability in real samples such as blood serum.

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P-818

Mechanical characterization of phase separated FG particles using Atomic Force Microscopy techniquesJ. Dietz¹, A. Janshoff¹, S. C. Ng², D. Görlich²¹Georg August University Institute for Physical Chemistry, Germany; ²Max Planck Institute for Biophysical Chemistry, Germany

While keeping nuclear and cytoplasmic contents separated, protein complexes—like the nuclear pore complex (NPC) of eukaryotic cells—conduct massive transport mediated by shuttling nuclear transport receptors (NTR) across the nuclear envelope.

Aqueous Nup98 FG domain solutions rapidly phase-separate into characteristic assemblies, called FG particles, on the micrometer scale; these are likely to be driven by multiple weak interactions involving interplay among electrostatic, dipolar, and short-range directional interactions. We study this particular soft matter that presents as a hydrogel using atomic force microscopy techniques (AFM) allowing us to reveal its intrinsic mechanical characteristics such as stiffness or viscoelastic behavior under load while simultaneously obtaining topographical information. We use force-clamp force mapping to measure the viscoelastic creep behavior, using sub-micrometer spatial resolution by combining force-distance curves with an added force clamp phase during tip-sample contact in which quantitative viscoelastic sample properties are extracted. Besides adhesion upon retraction we aim to implement viscoelasticity in the analysis of conventional force distance curves by fitting the entire force cycle.

Posters

– 26. Applications in biomedical and materials science –

P-819

Plant mutation breeding with heavy ion irradiation at IMP

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It is well known that heavy ion irradiation is characterized by a high linear energy transfer and relative biological effectiveness, which is expected to increase mutation frequency and mutation spectrum. So far a variety of mutants induced by heavy ion beam have been reported at the Institute of Modern Physics (IMP), affiliated with the Chinese Academy of Sciences. Long Fu No. 2, the new variety of spring wheat, was successfully bred by heavy ion beam mutation breeding techniques, which has extended the planting field of 5.5×10^5 ha during the past several years with excellent agronomic characteristics. White-flowered wandering Jew is a perennial evergreen herb. However, the mutant has been seasonally exhibited green or pink-variegated leaf and maintained by vegetative propagation. Sweet sorghum is a potential useful energy crop characterized by a high photosynthetic efficiency and a high biomass- and sugar- yielding crop. After irradiation by heavy ion beam, an early-maturity mutant was acquired and the growth period had stably shortened for around 20 days in sweet sorghum. In addition, a large number of mutants were isolated from maize, cotton, castor, violet and pelargonium, respectively. In the future, our research will be further focused on interaction of heavy ion beam with plant mutants at the molecular and protein level.

P-821

Spectroscopic and electrochemical evaluation of bovine serum albumin-folic acid interactionM. Florescu¹, C. G. Chilom²

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The binding mechanism of folic acid (FA) to free and bound bovine serum albumin (BSA) was studied both in solution and adsorbed on gold substrate.

The decay of the fluorescence emission of free BSA, was monitored in the presence of FA in increasing concentrations. The mechanism of the FA binding to BSA was found to have a static character. The distance between the donor (BSA) and the acceptor (FA) was determined on the basis of the nonradiative energy transfer efficiency between donor and acceptor. The BSA fluorescence quenching mechanism was characterized as a combination of a static quenching and a nonradiative energy transfer.

The adsorption of BSA and BSA-FA complex on gold substrate in increasing concentrations of FA was studied by cyclic voltammetry and electrochemical impedance spectroscopy. Changes in redox current and corresponding parameters of equivalent electric circuit at gold-solution interface were used to evidence and interpret the biomolecular interaction.

In order to characterize the FA-BSA interaction, the affinity constant and the thermodynamic parameters of the interaction were determined. The values of these parameters put in evidence a moderate exothermic FA-BSA binding process, driven both by enthalpically and entropically contributions.

P-820

Analysis of antimicrobial properties of graphene using Raman spectroscopyJ. Ferguson¹, I. Passaris², M. van der Woude², Y. Hancock^{1,3}

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The potential for biomedical applications of graphene is vast, such as drug delivery, bio-sensing and phototherapy [1, 2]. When interfaced with pathogens, graphene has been shown to cause cell-death, however previous studies provide little insight into the biomolecular mechanisms for cell destruction [3]. To address this, we use Raman spectroscopy to assess the antimicrobial properties of graphene interfaced with *E. coli*. Raman spectroscopy involves the inelastic scattering of monochromatic light to provide a molecular fingerprint of matter, whose sensitivity enables detection of changes in cell composition. Here we aim to further recent work [4] by carrying out full quantitative analyses of the Raman spectra to assess the biomolecular changes of *E. coli* interfaced with graphene. Specifically, we determine how the graphene ‘senses’ the pathogen, as well as quantify biomolecular changes to the pathogen signatures. Through the study of lipid and protein signatures in the Raman spectra, the destruction of the cell membrane, which has recently been reported, has also been assessed.

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P-822

Quantifying STED images discriminates dynamic casein structures relating to rheology and processing

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Casein proteins are the main structural element in many fermented dairy products. Advances in Super Resolution Microscopy have provided novel tools to investigate protein microstructures. Herein, Stimulated Emission Depletion (STED) Microscopy has been used to image casein micelle aggregation, gel formation and network coarsening over time. Protein structures have been resolved to > 100 nm. Native skim milk and reconstituted skim milk powder were used to produce gelled systems. Gelation was induced either through rennet addition or acidification with GDL. Images have been quantitatively analyzed using radial autocorrelation. Label free Coherent Anti-Stokes Raman Spectroscopy (CARS) Microscopy was used to verify that the presence of fluorophore did not interfere with gelation. Image data has been directly correlated with texture analysis.

We demonstrate the ability to resolve casein micelle structures on the scale of a single micelle. STED has provided a significant advantage over standard confocal imaging in dynamic time lapse experiments. This work provides the basis for the use of STED microscopy to analyze other colloidal structures. We present a framework to extract meaningful information that directly relates to the macroscopic properties and functionality of the system.

Posters

– 26. Applications in biomedical and materials science –

P-823

Dissecting multivalent DC-SIGN/R-Glycan interactions using polyvalent glycan-Quantum Dots

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Multivalent protein-glycan interactions play a key role in viral/bacterial infections.¹ They initiate the pathogen-cell contact that ultimately leads to infection. Glycoconjugates can block such binding and the potency critically depends on their spatial match. A challenge here is lack of structural details of key cell surface multimeric lectins. We show quantum dot (QD)-capped with arrays of simple glycans are powerful probes here. First, we develop a highly efficient method to make polyvalent glycan-QDs. Then, we study their multivalent binding with the HIV/Ebola receptors, DC-SIGN/DC-SIGNR² via a FRET readout strategy for the 1st time.³ We find that the polyvalent glycan-QD not only enhances its DC-SIGN binding affinity by 4–6 orders of magnitude, but also affords an unprecedented >60 fold binding selectivity for DC-SIGN over DC-SIGNR,³ despite almost identical tetrameric structure. This is attributed to their different binding-site arrangements. The QDs potently inhibit a pseudo-Ebola virus infection of DC-SIGN expressing cells (low nM IC₅₀),³ suggesting an excellent potential as anti-viral agents.

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P-825

In vivo magnetic resonance spectroscopy in the study of breast cancer metabolism

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Breast cancer is a heterogeneous disease and factors like hormone receptor status, lymph node involvement and grade of the tumor influence the patient's outcome. During cancer progression, the metabolism of cells or tissues is altered. In vivo MR spectroscopy (MRS) has played an important role in the study of tumor tissue metabolism. Our MRS studies on breast cancer showed high levels of total choline-containing compounds (tCho) in malignant breast tissue compared to benign and normal breast tissues. Early breast cancer patients showed a higher tCho compared to locally advanced breast cancer patients. tCho is a composite peak from phosphocholine, free Cho, glycerophosphocholine and its increase during malignancy indicate increased synthesis of cellular membranes. High tCho is associated with increased membrane synthesis required for proliferation of tumor. tCho showed no association with the ER, PR or HER 2/neu status of patients. However, the tCho was lower in triple negative in comparison to non-triple negative and triple positive breast cancer patients. These results indicate the complex molecular mechanism of cell proliferation and the molecular heterogeneity of breast lesions as well as the differences in metabolism between various sub-types of breast tumors.

P-824

Productive biofilms: bacterial interaction with component surfaces

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Bacterial adhesion is a prerequisite for productive biofilm formation. Scanning force -(SFM), lateral force microscopy (LFM) and single cell force spectroscopy (SCFS) were utilized to quantify lateral and vertical single cell adhesion forces of the gram negative seawater bacterium *Paracoccus seriniphilus*. Because pH and ionic strength can alter biofilm formation, these studies were conducted at different pH values and salt concentrations. The nanomechanics of the cell are mainly related to their turgor pressure. At pH 4 the results point to an active adaption mechanism to either protons or chloride ions, whereas the results at pH 11 show that the bacteria cannot adapt to alkaline conditions. In SCFS the vertical adhesion force of a single cell is measured. Alkaline pH results in a decreased force, probably due to damaged cell wall functions. For pH 7, where the bacteria grow best, the highest adhesion force was measured, which is beneficial for biofilm development. Furthermore productive biofilms were also grown in a biofilm flow reactor. Hence also lateral detachment forces were measured. The required force at which the bacteria start to detach strongly depends on the used surface. Higher roughness increases the required forces to move the bacteria in lateral direction.

P-826

Raman and SERS spectroscopic studies of [Ru(Phen)3]2+ and its photoproducts

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The determination of the oxygen partial pressure (pO₂) in real time in living biological tissues is of high interest for numerous therapies. Besides, assessment of the tissue oxygenation can also indicate malfunctioning of cellular metabolism. One interesting approach of its quantitative measurement is based on the oxygen-dependent fluorescence/phosphorescence quenching of molecular probes. Dichlorotris(1,10-phenanthroline)-ruthenium(II) hydrate (Ru[Phen]₃²⁺) is a promising sensitive pO₂ probe. However, its excitation also leads to the formation of the photoproducts which luminescence can induce perturbations in the pO₂ determination. Moreover, during the *in vivo* oxygen measurement the molecule of Ru[Phen]₃²⁺ can interact with various biomacromolecules what may affect its spectral properties, and thus, the determination of oxygen concentration. Raman and SERS spectroscopies were utilized here to achieve specific structural information of the Ru[Phen]₃²⁺ and its photoproducts and to dispose of an additional spectroscopic tool for their detection and further investigation.

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Posters

– 26. Applications in biomedical and materials science –

P-827

Analysis of functional segments in the coiled coil domains of yeast cargo receptors Emp46p/47pK. Kato¹, Y. Shimizu¹, E. Kurimoto¹, A. Oda^{1,2}¹Graduate School of Pharmacy, Meijo University, Japan;²Institute for Protein Research, Osaka University, Japan

Emp46p and Emp47p, which are yeast cargo receptors, form hetero complex through their coiled coil domain (Emp46c/47c) in a pH dependent manner. Emp46c/47c consist of two helix segments and a middle segment. A glutamate residue (E303) at the N-terminal segment of Emp46c is important for their assembly and mutations to this segment alter their pH profile. However, functions of the other segments are unclear. In this study, we downsized the sensor core of Emp46c/47c for application to various pH sensitive bio-devices. Proteins were expressed in *Escherichia coli* and hetero complex formations were analyzed using gel filtration chromatography. To extracted minimal functional segments, we examined whether C-terminal segments and middle segments of Emp46c/47c were required for hetero complex formation. We deleted C-terminal segments and analyzed their assembling properties. These mutants maintained pH-dependence similar to wild type. In contrast, substitution of flexible linker for middle segments impaired Emp46c/47c interaction. These results indicated that C-terminal segments were dispensable for their assembly although middle segments were required. Moreover, the hetero complex formation was detected by Förster resonance energy transfer using fluorescent protein fusion Emp46c/47c.

P-829

Probing supramolecular protein assembly using fluorescent molecular rotorsM. Kubánková¹, I. López-Duarte¹, J. A. Bull¹, D. Vadukul², L. Serpell², M. de Saint Victor³, E. P. Stride³, M. K. Kuimova¹¹Dept. of Chemistry, Imperial College London, London SW7 2AZ, UK; ²School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK; ³Institute of Biomedical Engineering, University of Oxford, Oxford OX3 7DQ, UK

We present a new optical approach for monitoring supramolecular assembly of proteins by following the fluorescence lifetime of environment-sensitive dyes termed Molecular Rotors (MR). The photophysics of MR depend upon the degree of crowding and microviscosity of their environment. We employed MR to study microrheological changes during protein aggregation, blood clotting, and live cell transformation upon the application of beta-amyloid protein, the aggregation of which is connected with Alzheimer's disease. In this last case we recorded two types of effects: (i) MR covalently attached to beta-amyloid proteins and (ii) MR selectively localised in the plasma membrane to study the change of bilayer microviscosity upon interaction of beta-amyloids. In all cases, significant change in MR fluorescence lifetime was observed during protein self-assembly. Our results demonstrate that monitoring the fluorescence lifetime of MR is a powerful tool for investigating the supramolecular assembly of proteins and the effects of such processes in vitro and in live cells.

P-828

Rotor-based organelle viscosity imagingM. Kubánková¹, J. E. Chambers², S. J. Marciniak², M. K. Kuimova¹¹Dept. of Chemistry, Imperial College London, London SW72AZ, UK; ²Dept. of Medicine, CIMR, University of Cambridge, Hills Road, Cambridge CB2 0XY, UK

Microscopic viscosity is a critical parameter in cellular processes such as diffusion-controlled reactions. A lack of a suitable technology has hampered the analysis of microrheology within the organelles of living cells.

We established a new technique called Rotor-based Organelle Viscosity Imaging (ROVI), in which fluorescent dyes termed Molecular Rotors (MR) are directed via active targeting to specific compartments of live cells for the dynamic measurement of microviscosity. The photophysics of MR depend upon the crowding and viscosity of their environment. Microviscosity can be quantitatively determined by measuring the fluorescence lifetime of MR via TCSPC FLIM.

Using ROVI, we measured microviscosity with a single probe in multiple cell compartments (cytosol, ER and mitochondria) basally and during manipulations that alter organelle function. We report striking variation of microviscosity between organelles and within matrices of mitochondria. Furthermore, we detected changes of ER microviscosity upon drug administration. The ability to measure the viscoelastic response of ER upon stress is novel fundamental science, highly pertinent to health and disease. We believe that ROVI offers novel avenues to study cell biology and to monitor the progression and treatment of diseases.

P-830

Combination of dielectrophoresis and SERS for bacteria detection and characterizationE.-M. Laux¹, A. Docoslis², C. Wenger³, F. F. Bier^{1,4}, R. Hölzel¹¹Fraunhofer IZI-BB, Germany; ²Queen's Univ., Canada;³IHP GmbH - Leibniz Institute for Innovative Microelectronics, Germany; ⁴University of Potsdam, Germany

Electrode arrays are used for the structured collection of *E. coli* bacteria at individual ring-shaped nanoelectrodes (20 nm ring width, 500 nm diameter). Bacteria are attracted to the electrodes under the influence of an inhomogeneous electric AC field. Dielectrophoresis, which is the dominant effect of the electric field applied here, causes a movement of the bacteria towards the electrodes and hence a concentration effect. Cells of *E. coli* are captured out of suspensions; both temporal and permanent adhesion of cells is achieved depending on electric field parameters.

Bacteria are detected by surface enhanced Raman spectroscopy (SERS), where the surface enhancement is introduced by the addition of silver nanoparticles (AgNPs, 50 nm diameter). Four different strategies for AgNP decoration of the electrodes, with and without electric fields, are compared. Spectral changes are used to characterize the interaction between AgNPs and bacterial cell components.

The use of nanoelectrode arrays with thousands of electrodes offers rapid analyses of large numbers of individual pathogens. The combination of a selective and accelerated target capture method (dielectrophoresis) with a sensitive signal transduction technique (SERS) is promising for the characterization of pathogens like bacteria or virus particles.

Posters

– 26. Applications in biomedical and materials science –

P-831

Towards artificial light harvesting antennas

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The mechanism that enables biological systems to efficiently capture and transduce light energy is still not fully understood, despite the intense research focus. Here is presented a highly flexible and customisable model light harvesting system for the study of the fundamental principles of biological light harvesting, emphasising some of the most distinct features of biological systems: establishment of energy gradients for energy transduction, precision of chromophore placement and system homogeneity. The model system is based on protein nanofibres comprised of idealised modular tetratricopeptide repeat (TPR) proteins; the TPR proteins provide a regular ordered surface suitable for the display of chromophores with desired spacing and density with atomic-resolution precision, thus allowing to control the extent of chromophore conjugation and to mimic biological arrangements. Individual TPR modules are functionalised with porphyrin chromophores and subsequently stepwise assembled into permanent supramolecular structures through unnatural amino acid-mediated cross-linking. The precise control over the assembly process results in homogenous assemblies, whereas the precise placement of the conjugated chromophores facilitates the establishment of energy gradients.

P-833

Electric field control of peptide-binding to biomaterial surfaces

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Implantable medical devices suffer from nonspecific protein adsorption from surrounding tissue causing adverse responses such as inflammation, infection, and rejection. Surface functionalization of an implantable device with bioactive molecules can overcome this by promoting biointegration, preventing the device being recognized as foreign. Here we report the control over the immobilization of surface-bound peptides by tuning electric fields at the surface. Guided by simulation, a peptide with a linear conformation in solution was designed. Electric fields were applied to control the approach of the peptide toward a radical-functionalized coating prepared by an energetic ion-assisted plasma process. Spontaneous, irreversible immobilization onto the coating was achieved through free-radical chemistry. We show that control of both surface concentration and orientation of the immobilized peptide is achieved simply by varying solution pH. We also demonstrate that an externally applied electric field is an efficient alternative to pH modulation to optimize peptide density and orientation. The findings show the mechanisms of peptide immobilization and have important implications for the design of synthetic biomolecules used for the development of modern bio-functionalized materials.

P-832

Determination of radioactivity in the propolis samples collected from Blacksea region in Turkey

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Propolis is a sticky substance that bees make which is better known as "bee glue". The process begins when an expert propolis-making bee gathers resin from cone-producing evergreen trees or from the buds of trees. It is being used as a natural food by the people. The aim of this study was to do the radioactivity analysis of propolis samples collected from towns of the ten cities at western blacksea region in Turkey when considering pollution agents and geographical and botanical factors. The propolis samples were collected from bee farms in cities of Blacksea region, determined by the sampling method. It has been 25 years since a disastrous explosion in Chernobyl nuclear power plant happened, yet its effects still continue to appear in the region, especially on the Black Sea coast of Turkey. At the end of the analyses, the most radioactive propolis samples and region were determined.

Keywords: Propolis, Bee Product, Radioactivity, Blacksea Region,

P-834

Brillouin microspectroscopy and Raman analysis for the study of amyloid plaques in transgenic mouse

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Amyloidopathy is one of the main hallmarks of Alzheimer's disease and it is characterized by the accumulation, in the brain parenchyma, of amyloid plaques, an extracellular deposit of amyloid- β (A β) protein aggregates. Here, we present the first application of micromechanical mapping based on Brillouin spectroscopy with correlative Raman scattering to amyloidopathy in a histological section of mouse brain containing the hippocampus. Brillouin and Raman microspectroscopies are contactless optical techniques, that give access to chemical composition and molecular structure of the tissue on a sub cellular scale. We show for the first time that amyloid plaques have a complex mechanical structure, with a rigid core of β -amyloid protein surrounded by a softer ring-shaped region richer in lipids and other protein conformations. These results are key to enable further developments towards the contactless diagnosis of amyloidopathy.

Posters

– 26. Applications in biomedical and materials science –

P-835

Philosophical biophysics and UNO-Agenda 21

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Introduction: Philosophy is *science of science* considering all disciplines acc to Immanuel KANT reflected by epistemology. Similar to medical and quantum philosophy (J HOGAN, CF von WEIZSAECKER 1992) is necessary creation of philosophical biophysics incl. A-C. A. EPISTEMOLOGY Acc to *axiology logic semantic* are to be clarified *notions in biophysics*: Volume&limits of application in biological, medical, psychological disciplines are to be clarified in context of scientific theory and integrative biophysics. B. MORAL PHILOSOPHY Independently from various ethical theories (deontology etc) acc to KANT conc a priori *human obligations to himself* – example for better ethics of biophysicists (a), other *humans* – social responsibility (b), *sub-human* eg radioecology (c), *suprahuman beings* moral limits e.g. rad biophysics, IORT/intraoperative radiotherapy (d). C. AESTHETICS In relation to A-B is necessary interdisciplinary discussion about side effects by application in medical physics e.g. photo&radiotherapy.

Conclusion: Common Congr. sessions of IUPAB, EBSA, etc with philosophical (FISP ISB etc) psychol (IUPsyS etc) physiol (IUPS etc) medical soc (FIGO ISIM etc) could open new dimension in biophysics in context of UNO-Agenda 21 for better health ecology etc on global level.

P-837

Structural studies of calixarene-mediated noncovalent PEGylation

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Noncovalent PEGylation¹ has great potential to increase the size, solubility, and stability of therapeutic proteins. Noncovalent strategies avoid chemical modifications which can be costly and time-consuming (purification) and may eliminate the functional impairment that can occur in conventionally PEGylated therapeutics. Calixarenes are a versatile class of macrocyclic compound with many advantages over other host systems due to their easily-modified upper and lower rims². Protein recognition by calixarenes has received significant attention in recent years. Sulfonato-calix[4]arene (sclx₄) has been shown to encapsulate lysine and arginine side chains of cationic proteins^{3,4}. Here, we have synthesized and characterized mono- and di-PEGylated sulfonato-calix[4]arenes and performed structural studies of their interactions with the model protein, cytochrome *c*. An unprecedented crystal structure (at 2.70 Å resolution) of a PEGylated-calixarene bound to cytochrome *c* will be presented.

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P-836

Capsulight: an innovative phototherapeutic strategy against severe gastric infections

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Conventional antimicrobial strategies have become increasingly ineffective due to the emergence of antibiotic resistance among pathogenic bacteria. In order to overcome this problem, antimicrobial PhotoDynamic Therapy (PDT) is considered an alternative promising therapy. PDT has a broad spectrum of action and low mutagenic potential and is particularly effective when microorganisms present endogenous photosensitizing pigments. *Helicobacter pylori* (Hp), a pathogen notoriously responsible for severe gastric infections (chronic gastritis, peptic ulcer, MALT lymphoma and gastric adenocarcinoma), might be a suitable target of antimicrobial PDT as produces and accumulates the photosensitizers Protoporphyrin IX and Coproporphyrin I. With the aim to design and develop an ingestible LED-based robotic pill for intragastric phototherapy so that irradiation can be performed in situ without the use of invasive endoscopic light, the action spectra for Hp photokilling will be determined. The photophysical properties of the Hp porphyrins will be studied. Hp culture samples will be irradiated at various wavelengths and intensities to determine the most efficient light conditions.

Framework project: “CapsuLight” (CUP B52I14005760002) financed by Regione Toscana, Bando FAS Salute 2014 (Italy)

P-838

An International Academy of Biophysics (IAB)

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Introduction: Globalization needs new models for better science, e.g. foundation of an IAB via network of selected nat. inst./univ. with common personnel (intern employees), possibility for whole life work, etc. AIMS OF IAB: To discuss philosophical fundamentals of biophysics: Its structure, relation to other disciplines in context of sci. theory, bioethics, etc. To develop model interdisciplinary programmes with emphasis on participation from biophysics & basic sciences (physics, chemistry, etc) as well as intern. educational programmes for post graduate training in biophysics conc holistic & multidimensional approaches. Founding of an International Biophysical Academy, also to discuss possibility for help for developing countries. **Conclusion:** An IAB could support UNO-Agenda 21 for better health, ecology in all countries. DEDICATION for moral support 2017-1980 of Nobel Laureates: France: M Allais J Dausset J-M Lehn FRG: M Eigen H Michel E Neher Japan-USA: K Fukui L Esaki S Tonegawa GB: B Josephson Sir J Kendrew Lord A Todd Russia: N Basov A Prokhorov A Sacharov USA-India: P Anderson, J Deisenhofer, H Khorana L Pauling E Wiesel

Posters

– 26. Applications in biomedical and materials science –

P-839

Smart polymers as linkers in single molecule force spectroscopy

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The interdisciplinary field of biophysics, especially single molecule force spectroscopy (SMFS) benefits from improvements in physics, mathematics and biochemistry. Being able to observe and describe force-related protein mechanics on a single molecule level sheds light on otherwise hidden characteristics. Amongst other discoveries, new high force interaction partners i.e. the Cohesin Dockerin Type III was observed and described. With the advancement in the higher force range, enthalpic overstretching of polyethylene glycol (PEG), a standard linker for SMFS, occurs. In aqueous buffers, forces above 200 pN trigger conformational change of PEG from trans-trans-gauche to an all trans state, thus increasing the net length of the polymer. This ultimately corrupts determination of contour length increments between two unfolding events within and beyond this force regime in SMFS data sets. Here we tackle this issue by replacing PEG as spacer molecule with the smart biopolymer elastin polypeptide (ELP). Establishing a new cloning technique allowed us to create monodisperse ELPs with defined termini for adaptive bioconjugation, depending on the desired immobilization strategy. It is now possible to ligate the protein of interest to ELPs, generating a polypeptide with only amide bonds. This improves the overall data quality due to a more homogeneous stretching of the anchored protein.

P-841

Development of the Caf1 protein as a multifunctional biomaterial for use in 3D cell scaffoldsD. T. Peters¹, G. Dura², H. Waller¹, Y. Ulusu^{1,3}, D. A. Fulton², J. H. Lakey¹¹Institute for Cell and Molecular Biosciences, Newcastle University, UK; ²Chemical Nanoscience Laboratory, Newcastle University, UK; ³Department of Bioengineering, Karamanoglu-Mehmetbey University, Turkey

The growth of mimetic tissues often requires the use of a 3D scaffold. An ideal scaffold would be bioactive, stable, and resemble native extracellular matrix (ECM) proteins. Caf1 is a promising new biomaterial that consists of um long protein polymers of 15 kDa subunits. *In vitro*, cells adhere poorly to Caf1, so new functions can be engineered in to control cell adhesion, differentiation and proliferation. Caf1 polymers show remarkable stability, can be produced economically by bacterial fermentation, and can form a porous 3D hydrogel when mixed with chemical crosslinkers. These properties highlight the potential for using Caf1 as a scaffold biomaterial. Here, we determine the thermostability of Caf1 polymers in a variety of harsh chemical conditions. We then investigate the rheological properties of the polymer in solution, showing they exist as a complex viscous liquid that is compatible with 3D bioprinting technologies. Finally, we use synthetic biology techniques to combine engineered Caf1 subunits and form mosaic Caf1 polymers that mimic ECM proteins. These results pave the way for the production of animal free, 3D printed, stimuli-responsive Caf1 hydrogels of definable bioactivity, which could find applications in drug discovery, 3D tissue culture and wound healing.

P-840

Advanced solid state characterization of polymorph: surface properties and its impact on dissolutionB. F. C. Patricio¹, L. D. Prado¹, K. M. Gonçalves¹, G. M. Rocha², G. Weissmüller², P. M. Bisch², H. V. Rocha¹¹Laboratory of Advanced Pharmaceutical Systems – Farmanguinhos/Fiocruz, Brazil; ²Laboratório de Física Biológica, IBCCF, UFRJ, Brazil

Poorly water-soluble drugs have low bioavailability and poor oral absorption, so they are a challenge for pharmaceutical industry. The use of polymorphs is a way to achieve appropriate bioavailability and has been extensively studied. Depending on the groups exposed on the surface their dissolution can be changed. By doing high resolution images it is possible to better understand the surface morphology and its physical properties. These parameters have direct impact on the bulk processability. The goal of this work is to evaluate and characterize the polymorphous II and III of the drug carvedilol (CAR) and its dissolution. Carvedilol presents low solubility in water with limited bioavailability. We study the polymorphs II and III of this drug. The polymorph II plane (0 0 1) of CAR presented flat terraces separated by steps of 1.5 nm. It is comparable to the interplanar spacing and along the a-axis by the single crystal XRD analysis. Also, this polymorph has smaller adhesion, elasticity and roughness than polymorph III. Furthermore, it was evaluated the dissolution the polymorph II plane (0 0 1) in the pH of 6.8 and 4.5. The results to the (0 0 1) plane indicate that the dissolution occurred on the surface by the recession of the molecular steps (1.5 nm) in both medium.

P-842

***In vivo* / *ex vivo* EPR spectroscopy in the study of amyotrophic lateral sclerosis**A. Popović-Bijelić¹, A. Pavićević¹, S. Stamenković², P. R. Andjus², M. Mojović¹¹EPR Laboratory, Faculty of Physical Chemistry, University of Belgrade, Serbia; ²Center for laser microscopy, Faculty of Biology University of Belgrade, Serbia

The biochemical mechanisms associated with the pathology of amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder, involve a toxic gain of function of mutant Cu,Zn superoxide dismutase (SOD1), its intracellular aggregation, as well as a complex interaction between redox active metals and reactive oxygen and nitrogen species (ROS/RNS). Here we review how *in vivo* and *ex vivo* electron paramagnetic spectroscopy (EPR) can be successfully used to study the ALS-induced changes of the redox status, and the blood-brain barrier (BBB) permeability in the SOD1G93A transgenic rat model, with the aim to elucidate the disease pathogenesis, and to find reliable biomarkers for early diagnosis and assessment of treatment outcome. *In vivo* studies, using aminoxyl radicals with different cell membrane permeabilities, show that the brain redox status and BBB are already disrupted in the presymptomatic stage of the disease. *Ex vivo* studies of brain tissue homogenates indicate an increased content of oxidized mitochondrial iron-sulfur clusters, uncoupling of heme-iron and copper in the O₂-reduction site of cytochrome c oxidase, as well as increased amounts of manganese in the spinal cord and brainstem. Future experiments will involve *in vivo* EPR spintrapping of ROS/RNS.

Posters

– 26. Applications in biomedical and materials science –

P-843

2D/3D CNT-based platforms for enhanced neuronal network developmentI. Rago^{1,2}, L. Cozzarini², R. Rauti³, A. Goldoni², L. Casalis², L. Ballerini³, D. Scaini³¹University of Trieste, Physics Department, Italy; ²ELETTRA Synchrotron Light Source, Nano-Materials Lab, Italy; ³International School for Advanced Studies (SISSA), Neurobiology, Italy

The application of nanotechnology to cell biology has opened promising strategies for nerve tissue recovery. In particular, carbon nanotubes (CNTs) are becoming relevant in neurobiology. CNTs outstanding electrical conductivity combined with dimensions comparable to extracellular matrix fibrils, a proved *in-vitro* and *in-vivo* cellular biocompatibility pose them as smart tools to specifically interact with CNS' cells at the nanoscale. In this framework, CNTs ability to boost neuronal activity is particularly intriguing. Here, we investigate the interaction between CNTs forests, directly grown on 2D/3D scaffolds through catalytic chemical vapor deposition (CCVD), and cultured primary neurons, to develop a reliable approach to drive neuronal network development. Morphological (confocal and scanning electron microscopy) and functional measurements (Ca²⁺ imaging) performed on these CNTs scaffolds-neurons nano-hybrids revealed that neurons adhere well to CNTs, extending their neurites and creating intimate and tight contacts with them. Remarkably, our CNTs substrates can effectively improve neuronal electrical performance. The versatility of our synthesis method allows to design more complex CNT-based synthetic biomaterials with 2D/3D architectures for neuro-medicine applications.

P-845

Assembling engineered proteins on gold nanoparticles for biosensing applicationsT. Robson¹, D. Shah², J. H. Lakey¹¹Institute of Cell and Molecular Biosciences, Newcastle University, UK; ²Orla Protein Technologies, UK

Nanoparticles are widely used in sensing applications and are advantageous for several reasons. They exhibit unique physical phenomena such as tuneable fluorescence (quantum dots) and local surface plasmon resonance (gold nanoparticles). Good sensitivity can also be achieved due to the high surface area to volume ratio of these materials. Gold nanoparticles are of particular interest as they can be readily made in various sizes and geometries; and can be functionalised with a number of different surface chemistries.

The Industrial CASE sponsor of this project, Orla Protein Technologies, utilise the self-assembling nature of the *E. coli* outer membrane protein A to generate well-ordered protein arrays on gold surfaces. This robust scaffold protein can then be engineered with a wide variety of functional motifs or domains such as cell adhesion proteins, single chain antibodies, or antigens. It has previously been shown that this system can be used for label free detection of an Influenza A antigen on flat gold surfaces. Here, we demonstrate the successful transfer of this technology to the surface of gold nanoparticles with the protein functionality maintained, allowing the novel use of engineered recombinant proteins in platforms such as lateral flow assays.

P-844

NMR spectroscopic analysis of protein-surface interactions – The effect of PEGylation

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PEGylation is a versatile means to increase the size, stability and solubility of protein therapeutics. Although PEGylation is a mainstay of the biopharmaceutical industry, structural knowledge of PEGylated proteins is relatively lacking. Few NMR characterizations exist¹⁻⁴ and just recently the first crystal structure of a PEGylated protein was reported.⁴

Here, we present an NMR structural investigation into the effect of PEGylation on protein recognition events, using the lectin RSL as a model system. RSL is a ~29 kDa trimer with each monomer containing four conjugation sites for NHS-ester PEGylating reagents. The preparation of mono-, di-, and tetra-PEGylated RSL species was facilitated by mutagenesis.

The interactions of PEGylated RSL species with the globular polysaccharide Ficoll 70 were analysed by NMR providing insight into how the degree of PEGylation and position of the PEG chains can impact binding.

References

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P-846

Adsorption/interaction of sugars and proteins: For a better understanding of biofilm formationC. Rösch¹, K. Huttenlochner¹, J. Gertje¹, F. Mahler¹, S. Trautmann², N. Umanskaya², C. Müller-Renno¹, M. Hannig², C. Ziegler¹¹Dep. of Physics; Univ. Kaiserslautern; Kaiserslautern, Germany; ²Dep. of Operative Dentistry and Periodontology; Univ. Hospital Saarland; Homburg, Germany

Biofilm formation is a complex phenomenon involving several biomolecule types: proteins, sugars, lipids, nucleic acids and bacteria. The first step of biofilm formation is the formation of a conditioning film of non-bacterial components that bacteria will later on colonize. Here, adsorption and interplay of proteins and sugars in the light of biofilm formation is investigated. A multimethod approach of Quartz Crystal Microbalance and Bicinichionic acid/Phenol sulfuric acid Assays can determine both, absolute amount of adsorbed biomass and adsorbed amounts of proteins/sugars. In addition, Scanning Force Microscopy can identify layer thickness. Dextran as common carbohydrate biofilm component and the protein albumin are used. The amount of adsorbed dextran increases with molecular weight and as for albumin shows a dependency on pH and surface material. Dextran exhibits smaller amounts and layer thicknesses on surfaces than albumin. Simultaneous adsorption of dextran and albumin leads to a cooperative adsorption. Consecutive adsorption induces partial displacement effects and adsorption of the second molecule species on/in the primary adsorbed layer. In conclusion, there exists a complex interplay between the components, which determines biofilm formation.

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– 26. Applications in biomedical and materials science –

P-847

Characterization of AfIV and fullerene C₆₀ interaction using atomic force microscopyY. Rud^{1,2}, Y. Prylutskiy¹, L. Buchatsky^{1,2}¹ESC Institute of Biology, Taras Shevchenko National University of Kyiv, Ukraine; ²Institute of Fisheries of the National Academy of Agrarian Sciences, Ukraine

Viruses cause many serious diseases in humans, animals and plants. The long-term side effects, low effectiveness of standard pharmacotherapy and the emergence of drug resistance require a search for new alternative or complementary antiviral therapeutic approaches. One new approach to inactivate microorganisms is photodynamic antimicrobial therapy.

Fullerenes and their derivatives are able to penetrate through cell membranes and they have strong antioxidant, antiviral and antimicrobial properties. Thus, the purpose of this work was to study the effect of photoactivated C₆₀ on virus titer using atomic force microscopy (AFM). Iridovirus of mosquito *Aedes flavescens* (AfIV) and multi host wax-moth larvae *Galleria mellonella* were used as model system.

In this report we demonstrate that the water-soluble fullerene (C₆₀) can be used to mediate the inactivation of iridovirus. Solution containing C₆₀ plus AfIV was illuminated with visible light for up to 1 h, resulting in a loss of infectivity in wax-moth larvae *Galleria mellonella* of more than 4.5 lg ID₅₀/ml. For these reasons, C₆₀ may prove useful in the inactivation of iridoviruses in biological systems as was shown by the AFM.

P-849

Coiled-coil based hydrogels as tunable scaffolds for investigating cellular mechanosensingA. Sanz de Leon, M. Goktas, P. Lopez Garcia, K. G. Blank
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Coiled coils (CCs) are highly abundant motifs in structural proteins. Consisting of two (or more) α -helices wound around each other in a superhelical fashion, they represent essential structural elements of the cytoskeleton and the extracellular matrix (ECM). Synthetic CC sequences are further used as dynamo-mechanical crosslinkers in biomimetic hydrogels. However, surprisingly little is known about the structural determinants that define the molecular mechanical properties of CCs.

Using AFM-based single molecule force spectroscopy, we have established the sequence-structure-MECHANICS relationships of a series of synthetic CCs. We show that the molecular rupture force of CCs depends on CC length and helix propensity as well as on the pulling geometry. Based on this knowledge, we have developed a library of CC sequences with tunable mechanical properties and synthesized a series of poly(ethylene glycol)-based hydrogels using these CCs as crosslinkers. The resulting hydrogels consist entirely of mechanically characterized molecular building blocks and allow for establishing a direct relationship between molecular and bulk mechanics. Using this series of hydrogels as an ECM mimic will allow for dissecting local and global factors that determine the process of cellular mechanosensing.

P-848

The NMR quantification of propolis constituents from North region of TurkeyZ. Safi Oz^{1,1}, M. Maras Atabay^{2,2}, M. Ferah Akpolat^{3,3}, K. Gulle^{3,3}¹Bulent Ecevit University, Faculty of Medicine, Department of medicinal biology, Kozlu, Zonguldak, Turkey; ²Bulent Ecevit University, Faculty of Education, Department of Mathematics and Science Education, Kdz Ereğli, Zonguldak, Turkey; ³Bulent Ecevit University, Faculty of Medicine, Department of Histology and Embriology, Kozlu, Zonguldak, Turkey

Propolis contains arich variety of chemical compounds. The chemical composition of propolis varies significantly depending on the specific plant resin used by the honeybees in propolis production. Multivariate analysis of data from propolis extracts has proven to be useful technique to show differences among propolis components. An NMR approach for quantification of the most studied is a rapid, easy to perform, reliable method for highly bioactive poplar propolis constituent. North and cost propolis differ significantly from South. Differences were observed in the qualitative and quantitative values of constituents in the propolis from North region of Turkey.

P-850

Colloidal quantum dots as a tool to track reversible binding of thylakoid proteinsK. Sołtysiak¹, K. B. Gieczewska², M. Łazicka², J. Grzyb¹¹Institute of Physics PAS, Warsaw, Poland; ²Faculty of Biology, University of Warsaw, Poland

Quantum dots are luminescent nanocrystals with already proved broad range of applications in life sciences and medicine – mainly as labels of proteins and other biomolecules. It was also shown that QDs may interact with photosynthetic electron transfer chain, by photoreduction of ferredoxin (Fd) and changing enzymatic performance of ferredoxin:NADP+ oxidoreductase (FNR). Here we are showing how such QD-Fd and QD-FNR hybrids interact with isolated thylakoids. Using confocal microscopy techniques we are proving that in presence of Fd or FNR binding to thylakoids was faster. This suggests specific interactions of QD-Fd and QD-FNR with those protein's binding sites. We are showing that binding is pH-dependent. We are also analyzing the QDs and QD-Fd/QD-FNR influence on thylakoid membrane model (namely liposomes containing LHClI only) in order to understand the unspecific binding and fluorescence energy transfer events in more details. The results are discussed in the perspective of QD-protein conjugates application in photosynthetic research and biotechnology. Research supported by National Centre for Research and Development (Lider/012/445/L-4/12/NCBR/2013) and conducted in NanoFun laboratories POIG.02.02.00-00-025/09.

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– 26. Applications in biomedical and materials science –

P-851

Blood-brain barrier disruption, redox status and elemental composition in the brain of the transgenic rat model of ALSS. Stamenković¹, M. Jovanović¹, M. Daković², V. Selaković³, P. R. Andjus¹¹Center for laser microscopy, Faculty of Biology, University of Belgrade, Serbia; ²Faculty of Physical Chemistry, University of Belgrade, Serbia; ³Institute for Medical Research, Military Medical Academy, Belgrade, Serbia

Amotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting motor and cognitive domains of CNS, characterized by neuroinflammation with blood-brain barrier (BBB) disruption, T cell infiltration, and disturbed redox status and elemental homeostasis. We studied the BBB integrity by MRI, tissue redox status by biochemical assays and tissue elemental composition by X-ray fluorescence imaging (XRFI), in the brain of the hSOD1^{G93A} rat model of ALS, in pre- (preALS) and symptomatic (sALS) animals. MRI with a G-based contrast and anti-CD4 or CD8 antibodies cross-linked with magnetic nanoparticles of iron oxide (MACS®) showed BBB leakage and T cell infiltration already in preALS. Biochemical assays revealed increased nitration, superoxide production, lipid peroxidation and MnSOD activity, and a decreased Cu-Zn SOD activity in brainstem and hippocampus homogenates of preALS and sALS animals. Finally, XRFI revealed a decrease of P and increases of Ca, Cl, K, Ni, Cu and Zn in the brainstem, and a rise of Cl, Ni and Cu, but a drop of Zn in the hippocampus of sALS animals. These results bring new biophysical insights into pathophysiological mechanisms and markers of ALS.

P-853

The nanoconstruction site at work – heterodimerizing helices as a tool to get nanocrystals togetherO. Sztatelman¹, J. Grzyb^{2,3}, K. Kopeć², M. Pędziwiatr²¹Institute of Biochemistry and Biophysics Polish Academy of Sciences, Poland; ²Institute of Physics Polish Academy of Sciences, Poland; ³Faculty of Biotechnology, University of Wrocław, Poland

There is a growing interest in optimizing the way to obtain nanobiohybrids in strictly controlled ways. Such nanobiohybrids usually are a combination of a nanocrystal and a biomolecule, and may have a broad range of applications: from a neutral fluorescent label only to a new functionality, resulting from cross-talk between nanohybrid partners. Here we present a simple system to combine more than two molecules in a nanohybrid. First, we got a fluorescent protein (GFP or mCherry) into a hybrid with a cadmium telluride quantum dot (QD) by simple electrostatic binding. Then, we combined such QD-proteins by interaction between heterodimerizing helices, present at the C-terminus of the proteins as an additional tag. As an option, we introduced cysteine bridge between helices, to stabilize complex. We confirmed the correctness of the protocol by analysis of conjugate's size and fluorescent properties. We also showed that the system may be used for controlled immobilization on the solid substrate surface. The possibility of further application is discussed.

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P-852

Dielectrophoretic functionalization of nanoelectrode arrays for the detection of influenza virusesS. Stanke^{1,2}, C. Wenger³, F. F. Bier^{1,2}, R. Hölzel¹¹Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalysis and Bioprocesses IZI-BB, Potsdam-Golm, Germany; ²University of Potsdam, Germany; ³IHP GmbH, Leibniz Institute for Innovative Microelectronics, Frankfurt/Oder, Germany

The influenza virus, as one of the major pathogenic agents for humans, is still a health threat. Consequently, a fast and simple characterization of viruses and virus subtypes is of great biomedical interest. The present work demonstrates the detection and characterization of influenza viruses via functionalized nanoelectrode arrays. Functionalization with antibodies is achieved by purely physical means applying AC electric fields with a strong gradient that leads to attraction to and permanent immobilization at the electrodes by AC electrokinetic forces (dielectrophoresis and AC electroosmosis). Immobilization is interpreted as a consequence of van der Waals and entropic forces. Since the electrodes are addressed individually, each can be functionalized with a different antibody. Each electrode is part of an on-chip resonant circuit, whose frequency changes with surface coverage of the electrode and, hence, serves as a measure of the amount of analyte bound to the antibody. For the detection of influenza virus particles additional dielectrophoretic attraction is utilized for locally concentrating these particles at the sensor surface. Owing to the universal principle of the system further application to other viruses and bacteria is envisaged.

P-854

Conformational heterogeneity in a fully-complementary DNA three-way junctionA. Toulmin¹, M. J. Morten², T. Sabir¹, L. E. Baltierra-Jasso^{1,2}, P. McGlynn³, G. F. Schröder⁴, B. O. Smith⁵, S. W. Magennis²¹The School of Chemistry, The University of Manchester, Manchester, UK; ²WestCHEM, School of Chemistry, University of Glasgow, Glasgow, UK; ³Department of Biology, University of York, York, UK; ⁴Institute of Complex Systems (ICS-6), Forschungszentrum Jülich, Germany; ⁵Institute of Molecular, Cell & Systems Biology, University of Glasgow, Glasgow, UK

Branched structures of nucleic acids are widely observed in nature as intermediates during DNA repair, recombination and replication, as well as being key components of DNA nanostructures. Using high-resolution single-molecule Förster resonance energy transfer (SM-FRET), we recently showed that a DNA three-way junction (3WJ) was not fully paired at the branchpoint, in spite of having a fully complementary sequence [1]. Here, we report the results of SM-FRET experiments alongside molecular dynamics simulations and the first use of ¹⁹F NMR on a fully complementary 3WJ, which shows that a 3WJ can adopt at least two major conformations, depending on the branchpoint sequence. This structural diversity has implications for processing 3WJs *in vivo* and their use in nanodevices.

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– 26. Applications in biomedical and materials science –

P-855

Bactericidal biocompatible moth eye mimetic nanopatterned polymer nanocomposites

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The propagation of multidrug resistant bacteria has prompted the need for radically different approaches to combat bacterial infection. Among these, natural inspired topographies¹ and nanocomposites² have emerged as effective non-resistance causing strategies to fight bacterial infection. In this work, we present moth eye mimetic nanopatterned polymer TiO₂ and ZnO nanocomposites fabricated by nanoimprint lithography. Our results exhibit enhanced antibacterial properties of TiO₂ and ZnO nanopatterned nanocomposites compare to nanopatterned polymer topographies or smooth nanocomposites against Gram positive and Gram negative bacteria, derived from the synergetic action of nanotopography and nanoparticle. Moreover, we assessed the biocompatible properties of ZnO nanopatterned nanocomposites and we showed off the great suitability of this material for cell development. Appearing as a promising material for implant devices and host tissue integration. Thus, here we present a promising technology for the development of bioinspired antibacterial materials with interesting application in the biomedical field and food packing, textile, shipping or furnishing industries.

¹Hasan et al. Trends Biotechnol. 2013, 31,1574²Palza, International journal of molecular sciences 2015,16, 2099

P-857

Mechanobiology of extracellular matrix: from 2D cell culture to cancer stroma

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Biophysical research revealed that extracellular matrix is far more than a scaffold to anchor cells. It controls the “rigidity” response of cells and regulates regenerative healing processes and, if missregulated, causes fibrosis and scarring. Animal models then showed that major transformations of extracellular matrix drive disease progression, including cancer, yet how the crosstalk between extracellular matrix and cells regulates these processes in tissues remains unknown. While many (nano)technologies have been developed in the last two decades to quantify the forces cells generate in cell culture and to probe how the stretching of proteins might switch their structure-function relationships, far less is known at the tissue level since no nanoscale force probes nor mechanical strain sensors are available so far to probe force-regulated processes in the complex 3D microenvironments of organs. We will discuss our most recent developments towards this goal as we have discovered a mechano-responsive peptide probe. Its affinity to FN fibers is regulated by the FN-fiber strain, and we validated this peptide as novel mechanical strain sensor in cell culture and in histological tissue sections from cancer stroma. Gaining insights how cells stretch ECM fibers at the tissue level is urgently needed since cells sense and respond not only to biochemical but also to physical factors in their microenvironments which co-regulate cell and tissue fate in homeostasis and disease.

P-856

Effect of the pulsed magnetic fields in *E. coli* and a theoretical model of growth populationP. Villegas¹, J. J. Bernal¹, T. Cordova¹, J. C. Martínez²¹Universidad de Guanajuato, Mexico; ²Instituto Politécnico Nacional, Mexico

Escherichia coli is a Gram negative enterobacteria and one of the most used microorganisms in the food and pharmaceutical industries; also, is used as laboratory model due to the easy manipulation and to its easily controllable growing conditions. They have reported the effects of magnetic fields on several microorganisms, particularly on *E. coli* using the frequencies in the range of 50–300 Hz. In this work, it was studied the growth population of *E. coli* submitted to magnetic stimulation in frequency series: 100, 800, 1500, 2450, 2500 Hz. In addition, a mathematical model was proposed for the total biomass growing and the bacterial growth rate, this one is a generalization of the classical exponential model, with the purpose of describe the cellular growth rate since the inoculation to the extinction. It was found that for a kinetic of the type:

$$N(t) = \frac{a_1}{1 + e^{-k_1(t-a)}} + \frac{a_2}{1 + e^{k_2(t-b)}} + C$$

It was obtained: $a_{1ENS}=10.083 \cdot 10^7$ cells/mL, $a_{1EPS}=10.94 \cdot 10^7$ cells/mL, $a_{1ECS}=13.56 \cdot 10^7$ cells/mL and $k_{1ENS}=5.051$ /h, $k_{1EPS}=3.459861$ /h, $k_{1ECS}=1.671$ /h. For the adjust of the kinetic experimental data to the model by minimum square method, the correlation factor R^2 was about 0.9. This results show a correlation between the application of the magnetic stimulation and the populational behaviour of the bacteria.

P-858

Manufacture of complex Caf1 protein polymers and hydrogel scaffolds for industry and medicine

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Here, we describe a new animal-free biomaterial with potential uses in 3D-tissue culture and regenerative medicine, due to its low cost, high stability and biodegradability. Caf1 is a polymeric protein from the plague bacterium *Yersinia pestis* which is secreted via the chaperone-usher pathway and protects the pathogen from phagocytosis by forming a non-stick protective layer around the cell. The 15.5 kDa monomer has an Ig-like fold and resembles the extracellular matrix protein fibronectin. The subunits polymerise via donor-strand complementation, forming a highly stable non-covalent polymer. Here we describe how we produce the recombinant polymer via batch fermentation using *Escherichia coli*. It is secreted by the bacterium into a flocculent layer above the cell pellet, and can be easily extracted and purified in large quantities. The polymer's high thermal stability, determined using SDS-PAGE and circular dichroism spectroscopy, confirms its robust character. Chemical cross linkers are then used to form stable 3D hydrogels with designed porosities. Additionally, we have selectively reversed the natural non-stick behaviour of the WT polymer by introducing an integrin binding sequence, RGDS, into the loop regions and can promote fibroblast adhesion to a hydrogel surface.

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– 26. Applications in biomedical and materials science –

P-859

Protein-nanocellulose bio-hybrid materials for advanced therapeutic applications

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Biomimetic materials engineered to elicit specific physiological responses have sparked increasing attention in both research and development. In this respect, the use of sustainable and non-toxic biopolymers such as plant-derived nanocellulose or microbial nanocellulose is of great interest. Antimicrobial peptides (AMPs) have emerged as a potent class of antibacterials in recent years due to their perceived advantages over traditional antibiotics such as the slower emergence of bacterial resistance. However the drawback is often related to their poor pharmacokinetics in solution and proneness to degradation. In the presented work aimed at conferring antimicrobial properties to nanocellulose, we combined the bacterial cell-wall-targeting peptide nisin with a matrix of TEMPO-oxidized nanocellulose (TONFC) as a new drug delivery system. The interaction with the small peptide molecules induced not only a dynamic rearrangement of the fibril network morphology, but also changed the interfacial properties of the fibrils drastically, as characterized by small angle X-ray scattering techniques. Surprisingly, a study of the antimicrobial activity of nisin-carrying TONFC revealed an enhanced antimicrobial potency of the immobilized nisin as compared to the soluble peptide.

P-861

Caffeine binds to antibiotics ciprofloxacin and tetracycline and alters their antibacterial activity

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Developing new ways of infectious diseases treatment is a major challenge for modern medicine. Caffeine, the most commonly consumed alkaloid worldwide, can reduce microbial growth. Due to its safety, it is a good candidate for combination antibacterial regimen.

Antibacterial studies showed that caffeine enhances or inhibits tetracycline and ciprofloxacin activity toward *Staphylococcus aureus*, depending on experimental conditions. UV-vis spectroscopy and isothermal titration calorimetry experiments showed direct interactions between antibiotics and caffeine with association constants $K_{AC}=36.8\text{ M}^{-1}$ (caffeine-tetracycline) and 39.9 M^{-1} (caffeine-ciprofloxacin), and enthalpy change $\Delta H=-2.9\text{ kJ}\cdot\text{M}^{-1}$ (caffeine-tetracycline).

Concluding, caffeine modulates antibacterial activity of studied aromatic antibiotics. Determined parameters for caffeine-antibiotic interactions suggest stacking complexes formation, which can explain decrease in antibiotic activity when both compounds are present in the medium. Observed, at certain conditions, enhancement of antibiotics antibacterial activity, leaves the door open for further studies on its application in clinics.

This study was financed by 2016/21/D/NZ7/01524 grant from National Science Centre, Poland.

P-860

Functional nanotubes: shape transformed polymersomes for biomedical applications

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Polymersomes are robust, versatile nanostructures that can be tailored by varying the chemical structure of copolymeric building blocks, giving control over their size, shape, surface chemistry and membrane permeability. In particular, the generation of non-spherical nanostructures has attracted much attention recently, as it has been demonstrated that shape affects function in a biomedical context. Until now, non-spherical polymersomes have only been constructed from non-degradable building blocks, hampering a detailed investigation of shape effects in nanomedicine for this category of nanostructures. Herein, we demonstrate the osmotically-induced elongation of spherical polymersomes comprising the biodegradable copolymer poly(ethylene glycol)-b-poly(D,L-lactide) into well-defined nanotubes. The size of these tubes is osmotically controlled using dialysis, which makes them very easy to prepare. To confirm their utility for biomedical applications, we have demonstrated that, alongside drug loading, functional proteins can be tethered to the surface utilizing bio-orthogonal 'click' chemistry. In this way the present findings establish a novel platform for the creation of biocompatible, high-aspect ratio nanoparticles for biomedical research.

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P-862

The potential application of FeTPPS in hemin therapy

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There has been a growing interest in the potential therapeutic applications of hemin and systemic treatment with hemin was protective in multiple acute injury models against ROS. However, Free heme damages lipid, protein and DNA through the generation of ROS which has been suggested to promote cell lysis and death.

5,10,15,20- tetrakis(4-sulfonatophenyl) porphyrinato iron (III) chloride (FeTPPS) is an artificial water soluble derivative of hemin, which has the same porphyrin ring and the same active site. FeTPPS has been used as a scavenger of peroxynitrite for inhibiting ONOO⁻ from causing nitration of tyrosine residues and antioxidant protects against oxidative injuries in many studies.

My studies found FeTPPS can effectively protect cells against ROS which is mediated at least in part by induction of heme oxygenase-1(HO-1) and itself is nontoxic. FeTPPS can binding to serum albumin with a very high association constant (about 10^8 M^{-1}). SA can decrease potential oxidative activity of FeTPPS and protect it against oxidative degradation, which means SA can assist in the transport and distribution of FeTPPS, effectively. So I explored the potential: FeTPPS replace hemin in hemin therapy.

Posters

– 26. Applications in biomedical and materials science –

P-863**Laser controlled singlet oxygen generation in mitochondria to promote mitochondrial DNA replication**

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The copy number of the mitochondrial DNA (mtDNA) varies in response to the physiological environment surrounding the cell. Reports have shown that a certain level of ROS can promote mtDNA replication. Singlet oxygen ($^1\text{O}_2$) is one of the main ROS generated in biological systems. We employed a photodynamic system to achieve controlled mitochondrial $^1\text{O}_2$ generation. HeLa cells incubated with 5-aminolevulinic acid were exposed to laser irradiation to induce $^1\text{O}_2$ generation within mitochondria. Increased mtDNA copy number was detected after low doses of 630 nm laser light in ALA-treated cells. The stimulated mtDNA replication was directly linked to mitochondrial $^1\text{O}_2$ generation. The stimulated mtDNA replication was regulated by mitochondrial transcription factor A and mtDNA polymerase γ . MtDNA control region modifications were induced by $^1\text{O}_2$ generation in mitochondria. A marked increase in 8-Oxoguanine level was detected in ALA-treated cells after irradiation. HeLa cell growth stimulation and G1-S cell cycle transition were also observed after laser irradiation in ALA-treated cells. These cellular responses could be due to a second wave of ROS generation detected in mitochondria. In summary, we demonstrated that mtDNA replication can be stimulated by a photodynamic approach in mammalian cells.

Posters

– 27. Protein folding and assembly –

P-864 (O-162)

Dual function of the trigger factor chaperone in nascent protein folding

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Large proteins often require help from molecular chaperones to fold productively, even before the ribosome has finished their synthesis. The mechanisms of chaperone function remain poorly understood. Using optical tweezers to study the folding of nascent elongation factor G (EF-G), a model multi-domain protein, we find that the N-terminal G-domain folds robustly on the ribosome. The following domain II, in contrast, fails to fold efficiently. Strikingly, interactions with the unfolded domain II convert the natively folded G domain to a non-native state that readily unfolds. The two unfolded domains subsequently form misfolded states. Both the conversion of natively folded domains and non-productive interactions among unfolded domains are efficiently prevented by the nascent chain-binding chaperone trigger factor. Thus, our single-molecule measurements reveal an unexpected role for the chaperone: It protects already folded domains against denaturing interactions with parts of the nascent polypeptide that are not yet folded. Previous studies had implicated trigger factor in guiding the folding of individual domains, but interactions among domains had been neglected. Avoiding early folding defects is crucial, since they can propagate and result in misfolding of the entire protein.

P-866 (O-160)

Self-organizing amyloid in bacteriaD. E. Otzen¹, B. S. Vad¹, M. S. Dueholm², P. H. Nielsen², S. L. Rouse³, S. J. Matthews³¹iNANO, Aarhus University, Denmark; ²Aalborg University, Denmark; ³Imperial College, UK

Functional bacterial amyloid proteins (FuBA) serve multiple purposes such as surface adhesion, biofilm formation, enhanced surface hydrophobicity, extracellular casing and cell wall strengthening. FuBA are extremely robust, often surviving boiling SDS, and are evolutionarily optimized to form amyloid structures under a wide range of condition, though often natively unfolded as monomers. FuBA systems appear to rely on two central features:

(1) **Sophisticated export and assembly systems.** Cells involve a cohort of ancillary proteins to form a FuBA export-and-assembly system in the outer membrane. Our recent structure of the export channel suggests that these ancillary proteins sculpt the folding energy landscape with a combination of nucleating proteins and “folding slides”.

(2) **Sequence design to avoid uncontrolled aggregation.** FuBAs have variable number of imperfect repeats of around 20-40 residues predicted to form hairpin structures in a monomeric structure that recapitulates the overall amyloid fold but only folds upon self-assembly. Our mechanistic studies indicate that FuBA aggregation is driven by a primary nucleation nucleation process which avoids a secondary nucleation step, unlike pathological amyloid like A-beta. This avoids an uncontrollable auto-catalytic feedback loop.

P-865 (O-161)

Disorder-to-order transitions involved in secretion, folding and functions of a bacterial toxinD. P. O'Brien¹, A. Voegelé¹, S. Cannella¹, D. Durand², P. Vachette², S. Brier¹, D. Ladant¹, A. Chenail¹¹Institut Pasteur, CNRS, France; ²I2BC, CNRS, France

The adenylate cyclase toxin (CyaA) plays an essential role in the early stages of respiratory tract colonization by *Bordetella pertussis*, the causative agent of whooping cough. Once secreted, CyaA invades eukaryotic cells, leading to cell death. The cell intoxication process involves a unique mechanism of translocation of the CyaA catalytic domain directly across the plasma membrane of the target cell. In the low calcium environment of the bacterial cytosol, the C-terminal RD domain is an intrinsically disordered coil, appropriately sized for transport through the narrow secretion machinery. Upon secretion, the high calcium concentration in the extracellular milieu induces the refolding of RD, which likely acts as a scaffold to favour CyaA refolding. Due to its hydrophobic character, CyaA is known for its propensity to aggregate into multimeric forms in the absence of a chaotropic agent *in vitro*. We have recently showed that calcium binding and molecular confinement are critical for CyaA folding into a stable, monomeric and functional state. Overall, this data demonstrates the adaptation of bacterial RTX toxins to the diverse array of calcium concentrations encountered in the successive environments during the cell intoxication process.

P-867

Lipid dependent insertion of the human N-BAR domain into artificial sarcolemma monolayersA. Auerswald¹, T. Gruber², J. Balbach², A. Meister¹¹Institute of Physical Chemistry, Martin Luther University Halle-Wittenberg, Germany; ²Institute of Physics, Martin Luther University Halle-Wittenberg, Germany

The N-BAR domain of Bin1 (amphiphysin 2) from skeletal muscle contributes to the generation of transverse tubules by forming protein scaffolds on the sarcolemma membrane. Recently, we showed that N-BAR binding to large unilamellar vesicles as well as uniform membrane curvature generation critically depends on the lipid composition of the sarcolemma membrane (1). Here, we discuss the role of the individual sarcolemma lipids to N-BAR recruitment using binding experiments with lipid monolayers. Langmuir monolayers represent one-half of a lipid bilayer and provide an ideal model system for peripheral adsorption and insertion of proteins. In this study, we applied the Langmuir film balance technique, combined with epifluorescence microscopy, to follow the peripheral insertion of the human N-BAR domain into artificial sarcolemma membranes of systematically varied lipid composition. Our results indicate that the natural mixture of sarcolemma lipids is essential for efficient N-BAR binding through its N-terminal amphipathic helix. We underline the role of PI(4,5)P₂ to protein recruitment and the limits of the monolayer technique by discussing the role of cholesterol.

(1) Daum B, Auerswald A, Gruber T, Hause G, Balbach J, Kühlbrandt W, Meister A. (2016) *J. Struct. Biol.* 194:375.

Posters

– 27. Protein folding and assembly –

P-868

Diverse intermediates of apomyoglobin are zones of a common area of a conformational states diagram

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Spectral properties of sperm whale apomyoglobin were studied by CD and fluorescence techniques over a wide range of conditions. Collected experimental data allow constructing the diagram of conformational states in coordinates of pH, temperature and urea concentration. It was shown that intermediate states observed under various modes of denaturation of this protein belong to one and the same area of the conformational states diagram.

P-869

A minimal ATP binding domain forms a folding nucleus for Hsp70 proteinsD. Bauer¹, R. Jakob², G. Zoldak¹, M. Rief¹¹Technical University of Munich, Germany; ²University of Basel, Switzerland

The folding energy landscapes of large proteins often involve partially stable structures, that can have profound effects on the timescale of the native state formation. Here we investigated the folding pathway of the Hsp70 nucleotide binding domain of *E. coli* (E.c.NBD) and the folding incompetent yeast mitochondrial NBD (mtNBD) using single-molecule force spectroscopy. Both domains share a similar three-dimensional structure which consists of 2 lobes (I and II) and are further sub-divided in a/b; a highly conserved ATP binding site is located at the interface of both lobes. We found that in vitro mtNBD does not refold under any conditions while E.c.NBD folds regularly by a sequential mechanism involving two on-pathway folding intermediates. We found that first lobe IIb folds very rapidly followed by the folding of the complete lobe II region. Surprisingly, MgATP can bind at micromolar affinity to the folded lobe II and thus represents the minimal ATP binding domain. In collaboration, we resolved the three-dimensional structure of this minimal ATP binding domain which is identical to the full-length NBD. Next, we designed a chimeric variant of mtNBD, by replacing lobe II with the E.c.NBD lobe II. Grafting the bacterial lobe II turns the folding incompetent mtNBD into a folding competent protein.

P-870

Surface methylation profiles unravel protein conformation. An NMR approachE. M. Bernar¹, G. E. Gómez¹, C. Smal², M. Arán², J. M. Delfino¹¹Departamento de Química Biológica e IQUIFIB. Universidad de Buenos Aires. CONICET, Argentina; ²Fundación Instituto Leloir, Argentina

Solvent accessible surface area of the polypeptide chain plays a pivotal role in protein folding and interactions. However, this fundamental parameter eludes direct scrutiny. The reaction of the minute photochemical reagent diazirine (DZN) with polypeptides mimics water because of its size, and shows limited chemical selectivity due to the extreme reactivity of methylene carbene (MC). Detection of products by NMR is advantageous because it does not demand cleavage of the polypeptide. The extent of MC reaction along the surface of *E. coli* thioredoxin (TRX) was assessed. The dominant modification involves methylation of amino acid side-chains, as attested by the enrichment of the aliphatic region in ¹H-NMR spectra. ¹H,¹³C-HSQC spectra of TRX reacted with ¹³C-DZN reveal new crosspeaks corresponding to water-exposed methyl groups. ¹H,¹⁵N-HSQC spectra show the different impact of the reaction on backbone amide environments. The relative intensity of CH α spots is indicative of the extent of methylation at individual amino acid residues. Moreover, CH β , CH γ , CH δ crosspeaks pinpoint details on side-chain methylation. A fully consistent pattern emerges from both HN and HC regions. Collectively, the protein methylation profile provides a unique footprint on protein conformation.

P-871

Biophysical characterization of hemocyanin of the freshwater shrimp *Macrobrachium acanthurus*G. Bertini^{1,1}, G. H. Faria^{1,1}, L. Ramos^{2,2}, P. S. Santiago^{1,1}¹UNESP, Registro, Brazil; ²UNESP, Araraquara, Brazil

The aim of the present work is to study the effect of different pH values of the hemocyanin of the freshwater shrimp *M. acanthurus* (HcMa) by optical absorption, fluorescence and light scattering at 90° (LSI). Samples of HcMa at 1.63 mg/mL concentration were prepared in 30 mmol/L acetate-phosphate-borate buffer at pH range from 3.7 to 10.2, which were equilibrated for 2 h before measurements. The absorption spectra of the HcMa were characterized by two bands centered at 280 and 340 nm, associated with the aromatic amino acids and ligand-to-metal charge transfer band, respectively. In the spectra of optical absorption was observed that the band at 340 nm shows a maximum intensity around pH 4.5 and above 9.0. However, the band at 280 nm shows intensity around 1.0 throughout the pH range. The maximum fluorescence emission of tryptophan shifted from 333 to 322 nm in the pH range from 3.4 to 10.2, evidencing the presence of different accessibilities of tryptophan to the solvent. The LSI increased at pH 4.5 and in the range between 6.0 and 8.0, after which it decreased. Therefore, it is still not possible to accurately determine the isoelectric point of HcMa, suggesting an isoform around 4.5 and another in 6.0–8.0.

Posters

– 27. Protein folding and assembly –

P-872

Structure of toxic Oligomers from AB1–42 Fibrils probed at the Nanometer Scale by TERSS. Bonhommeau¹, D. Talaga¹, J. Hunel¹, S. Henry², C. Cullin², S. Lecomte²¹ISM, 351 cours de la Libération, 33400 Talence, France;²CBMN, allée Geffroy St Hilaire, 33600 Pessac, France

The toxicity of amyloids is a subject under intense scrutiny. Many studies link this toxicity to the existence of various intermediate structures prior to the fiber formation and/or their specific interaction with membranes. For the first time, natural Ab1–42 fibrils (WT) implicated in Alzheimers disease, as well as two synthetic mutants forming less toxic amyloid fibrils (L34T) and highly toxic oligomers (oG37C), are chemically characterized at the scale of a single structure by Tip-Enhanced Raman Spectroscopy. TERS is a powerful technique combining the high sensitivity of surface-enhanced Raman scattering (SERS) and the nanoscale lateral spatial resolution of atomic force microscopy (AFM). A careful examination of amide I and amide III bands allows us to clearly distinguish WT and L34T fibers organized in parallel β -sheets from the small and more toxic oG37C oligomers organized in anti-parallel β -sheets. [1]. The interaction between membrane models and A β _{1–42} peptides and variants (L34T, oG37C) were also investigated using various biophysical techniques. We established that toxic stable oligomeric form (oG37C) interacts strongly with membranes leading to its disruption [2].

[1] S. Bonhommeau et al. *Angew. Chem. Int. Ed.* 56, 1 – 5 (2017).[2] S. Henry et al. *Biomacromolecules* 16, 944-950 (2015).

P-874

Real-time label-free detection and sizing of protein molecules using a deep UV microfluidic platformP. Challa, P. Quentin, W. A. Maya, Z. Yuewen, C. A. Jacqueline, K. P. J. Tuomas
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For the study of many key biomolecules, including peptides and proteins, that have low fluorescence at visible wavelengths, typically requires the labelling of the species of interest with extrinsic fluorophores, a process which can be time-consuming, requires purification steps, and has the propensity to perturb the behaviour of the systems under study due to interactions with the labels themselves. As such, the exploitation of the intrinsic fluorescence of protein molecules in the deep UV is an attractive path to allow the study of unlabelled biomolecules, and devices fabricated out of materials that are incompatible with widely used soft-lithography techniques. Here, we develop a deep UV-LED platform that allows the visualisation in real time of unlabelled proteins within microfluidic channels fabricated into PDMS doped with carbon nanoparticles using soft-lithography. Using this platform, we demonstrate intrinsic fluorescence detection of proteins at nanomolar concentrations on chip and combine detection with micron-scale diffusional sizing to measure the sizes of proteins in solution. Our platform will allow probing protein function, conformational change and interactions *in vitro*.

P-873

Thermostabilization of Lysozyme in Trehalose Water Mixtures by Infrared Spectroscopy

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Thermal stability of lysozyme in trehalose water mixtures is analyzed by Infrared spectroscopy measurements carried out in the 400-4000 cm⁻¹ frequency range. We found that trehalose show effects on the hydrogen bond network of water and that they reduce the protein dynamic fluctuations thanks to the strengthening of the intermolecular O-H interactions in the hydrogen-bond network of solvent which in turn leads to a destabilization of the tertiary structure. The temperature behavior of the spectral features of the amide I band of Lysozyme, connected with the dihedral angles of the various secondary structures present in the protein, are analyzed in terms of spectral difference and wavelet analysis. The analysis for lysozyme in trehalose water mixtures reveals, in respect to lysozyme in water mixtures, a higher value of the inflection point temperature revealing an increased stabilization temperature range together with a higher thermal restraint value.

P-875

Structural insight into capsid assembly and viral infection of piscine betanodavirus

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Betanodaviruses cause massive mortality in marine fish species with viral nervous necrosis. The structure of a T=3 Grouper nervous necrosis virus-like particle (GNNV-LP) was determined at 3.6 Å resolution [1]. Each capsid protein (CP) shows three major domains: (i) the N-terminal arm, an inter-subunit extension at the inner surface; (ii) the shell domain (S-domain), a jelly-roll structure; and (iii) the protrusion domain (P-domain) formed by three-fold trimeric protrusions. We also determined structures of the T=1 subviral particles of (i) the delta-P-domain mutant; and (ii) the N-ARM deletion mutant; and (iii) the structure of the individual P-domain [2]. The P-domain reveals a novel Dx₂D motif asymmetrically coordinating two Ca²⁺ ions, and plays a prominent role in trimerization of CPs during the initial capsid assembly process. The flexible N-ARM appears to serve as a molecular switch for T=1 or T=3 assembly. Several hypervariable regions on the P-domain coincide with the protrusion surface associated with functionalities of the receptor binding and host-cell specificity. The structural studies together with biological assays enhance our understanding of the capsid assembly and viral infection by betanodaviruses.

References:

1. *Acta Cryst.* (2016) D72, 830-840.
2. *PLoS Path.* (2015) 11, e1005203.

Posters

– 27. Protein folding and assembly –

P-876

Metal ion co-factors sculpt the heterogeneity of conformational landscape in Superoxide Dismutase

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Superoxide Dismutase (SOD), is a key component of cellular anti-oxidant defence system. Some 100 mutations leading to misfolding and aggregation in human Cu-Zn SOD (SOD1) have been implicated in the fatal motor neuron disease Amyotrophic Lateral Sclerosis (ALS). We probed into the folding and aggregation landscape of SOD1 and its metal specific mutants to unravel the structural interplay of two extended loops (IV and VII) which house the metal co-ordination sites and the activity tunnel. Deploying an ensemble bio-physical approach followed by statistical modelling using cluster algorithms we could figure out a secondary organizational flip upon disruption of the global structure and the early formed non-native local architectural signatures with a co-factor induced intrinsic disorder to order transition. The differential aggregation profiles of protein forms reflect the ways metal co-ordination may impact the seeding events. The distances among the protein variants were found to define the roles of metal ion cofactors in deciding the structure. Adopting direct coupling analysis and the co-variation algorithms we have traced the fingerprints of SOD's evolution from its primeval form which could account for the catalytic potency exploiting the heterogeneous inner organization.

P-878

Interplay of protein interactions involved in miRNA-mediated gene silencing, as revealed by HDX MS

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One of the crucial steps of miRNA-mediated silencing of mRNAs is the recruitment of the CCR4-NOT deadenylase complex by the GW182 protein. On the other hand, silencing of mRNAs containing AU-rich elements requires the recruitment of CCR4-NOT by the tristetraprolin protein. Here, we show how GW182 and tristetraprolin interact with CNOT1, the scaffolding subunit of the CCR4-NOT complex. Hydrogen-deuterium exchange mass spectrometry experiments revealed perturbations of hydrogen bonding networks upon binding of the mostly natively unstructured GW182 silencing domain and tristetraprolin fragments to CNOT1(800-999). The kinetic and equilibrium changes in the H/D exchange of CNOT1 peptide fragments upon the complexes formation revealed a putative binding site for the GW182 silencing domain, and accompanying conformational changes. Supported by ERFD through the Foundation for Polish Science International PhD Projects Programme, NanoFun POIG.02.02.00-00-025/09 project, Preludium 6 UMO-2013/11/N/NZ1/02387, and UMO-2016/22/E/NZ1/00656 grants from the Polish NCN.

P-877

Changes in the structure of poly-L-lysine triggered by fluphenazine molecules

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The fluphenazine (FPh) molecule is one of the most promising lead structures of the twentieth century. Its biological activities are strongly related to interactions with proteins. In order to perform the character of structural modifications of proteins induced by FPh molecules, the poly-L-lysine (PLL) was used as an excellent system for modeling properties of naturally accruing proteins.

The changes in a secondary structure and an self-aggregation processes of PLL triggered by FPh were studied using Fourier transform infrared (FTIR), vibrational circular dichroism (VCD) spectroscopy, and transmission electron microscopy (TEM). Depending on the external physicochemical conditions, the presence of FPh promotes a formation of -helical or antiparallel sheet structures of PLL.

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P-879

Following the acid-induced unfolding of Human Serum Albumin

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The most abundant plasma protein, Human Serum Albumin (HSA), is known to undergo conformational transitions in acidic environment. To avoid buffer effects and correlate global and local structural changes, we developed a continuous acidification method and simultaneously monitored the protein changes by both small-angle scattering (SAXS) and fluorescence. The progressive acidification, based on the hydrolysis of glucono- δ -lactone from pH 7 to pH 2.5, highlighted a multi-step unfolding involving the putative F form (pH 4) and an extended and flexible conformation (pH < 3.5). The scattering profile of the F form was extracted by component analysis and further 3D modeled, suggesting the rearrangement of the three albumin domains in a more elongated conformation, with a partial unfolding of one of the outer domains at this intermediate stage. To test the stabilizing effect of fatty acids, here palmitic acid, we compared the acid unfolding process of albumin with and without ligand. We found that when binding the ligand the native conformation was favored up to lower pH values. Our approach solved the problem of realizing a continuous, homogeneous and tunable acidification with simultaneous characterization applicable to study processes triggered by a pH decrease.

Posters

– 27. Protein folding and assembly –

P-880

Biophysical characterization of the yeast GRASP: an amyloid protein with intrinsic disorderN. Fontana¹, R. Maldonado², L. F. S. Mendes¹, A. Costa-Filho¹¹Laboratório de Biofísica Molecular, Departamento de Física, Universidade de São Paulo, Brazil; ²Instituto Federal de Ciência e Tecnologia de São Paulo, Jacareí-SP, Brazil

The Golgi Re-Assembly and Stacking Proteins (GRASPs) have been implicated on structural function within the Golgi apparatus and non-conventional secretion. However, their role in each situation lacks clarification. Our work aims to help elucidate the structure-function correlation in the GRASP of *Saccharomyces cerevisiae* (Grh1). So far we have managed to do not only a biophysical characterization of Grh1, but also prove that it has regions of intrinsic disorder (IDRs). Our results show lack of cooperativity upon denaturation, a circular dichroism spectrum with disordered contribution, among other data to support the affirmation. Besides that, our group surprisingly found that Grh1 is an amyloid protein, structuring itself on beta-sheet rich fibers under certain conditions, what has been confirmed by fluorescence experiments with specific dyes and microscopy. This finding opens a new area to explore, in the general context of amyloid fibers, especially considering their close relation with intrinsic disorder. We have a number of techniques at hand, and putative molecular partners for Grh1 being expressed, which enable us to do an even more complete study of Grh1, what will contribute scientifically on three biologically important aspects: GRASPs, IDPs and amyloid fibrils.

P-882

A microfluidic platform for quantitative protein studiesT. W. Herling¹, P. Arosio¹, T. Müller¹, D. J. O'Connell², M. C. Bauer³, J. Persson³, U. Weininger³, S. Linse³, T. P. J. Knowles¹¹University of Cambridge, UK; ²University of College Dublin, Ireland; ³Lund University, Sweden

Non-covalent interactions play a vital role in protein structure and function, from the specific binding of a single ion to the formation of large biomolecular complexes. Critically, the central physicochemical parameters defining these interactions remain challenging to quantify. To address this challenge, we have explored the potential of microfluidics in non-disruptive biophysical assays to develop a quantitative electrophoresis technique. This strategy enables an absolute value for the electrophoretic mobility to be determined, providing insight into the size-to-charge ratio of the sample molecules. We have applied this technique to study both the physicochemical properties of proteins and their binding to species ranging from a single ion to large biomolecules. Our findings highlight the difference between the dry sequence charge and the effective charge of a protein in solution. We use changes in the electrophoretic mobility upon complex formation to probe the calcium dependence of ligand binding by calmodulin, a protein at the heart of calcium-mediated signal transduction. This strategy does not rely on particular properties of the sample molecules and thus provides a general and quantitative approach for probing protein binding equilibria in free solution.

P-881

Protein structure characterisation using network analysisW. P. Grant¹, S. E. Ahnert^{1,2}, C. J. Pickard^{3,4}¹Theory of Condensed Matter Group, Cavendish Laboratory, University of Cambridge, UK; ²Sainsbury Laboratory, University of Cambridge, UK; ³Department of Materials Science and Metallurgy, University of Cambridge, UK; ⁴Advanced Institute for Materials Research, Tohoku University, Japan

Protein structure is determined by the folding of a chain of bonded amino acids into a unique three-dimensional configuration. This configuration then determines the function of the protein. The complexity of the folded structure makes analysis of the set of solved protein structures computationally challenging, but such analysis may yield valuable insight into the link between the protein structure and function.

In this work, the structure of a protein is abstracted by conversion into a network, in which the nodes of the network correspond to the atoms of the structure, and an edge is generated if the corresponding atoms are within a certain distance. Pre-existing network analysis methods can then be used to investigate the bonding patterns which make up the structure. Specifically, community detection algorithms are employed to break the protein into highly-associated modules. These modules can then be analysed across sets of proteins to find new conserved units, or to investigate the topology of the protein.

P-883

Artificial assembly of the bacterial flagella motor protein FliG on DNA nanostructuresS. Hertel, C. W. Liew, M. A. Baker, L. K. Lee
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The bacterial flagella motor (BFM) is a 11 MDa protein complex, composed of 13 different component proteins, which powers the rotation of the bacterial flagella filament. It can rotate counter-clockwise or clockwise which determines whether the bacterium runs smoothly forward or tumbles randomly, respectively. A protein called FliG forms a ring in the rotor of the BFM, which is involved in the generation of torque and rotational switching. FliG exists predominantly as a monomer in solution but as a polymer in the assembled BFM. This study aims to control FliG assembly using DNA nanostructures as an artificial template to functionally and structurally characterize interactions in the FliG ring *ex vivo*. To template FliG multimers, DNA nanostructures with varying numbers of FliG monomer binding sites have been designed and preliminary experiments with biolayer interferometry and surface plasmon resonance indicate FliG-FliG interaction on these templates. Additionally, DNA origami structures have been built to shape the FliG polymer into a ring, which allows for characterization of FliG interactions in the ring using single molecule microscopy.

Posters

– 27. Protein folding and assembly –

P-884

Lateral opening in the intact β -barrel assembly machinery captured by cryo-EM

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Outer membrane proteins (OMPs) mediate the survival and pathogenicity of Gram negative bacteria. The biogenesis of these proteins however, presents problems as they must be transported to, inserted and folded correctly in the outer membrane in the absence of ATP. The β -barrel assembly machinery (BAM) complex is a ~203 kDa complex of five proteins (BamA-E) that enables the membrane insertion and folding of substrate OMPs on a physiological timescale. Despite available crystal structures the mechanism of this vital protein complex remains poorly understood. We have used structural and biochemical tools to probe the nature of BAM-assisted OMP folding. We present the first cryo-electron microscopy structure of the complex, at a resolution of 4.9 Å. This reveals the intact BAM complex with BamA in a laterally-open conformation, between the first (β 1) and last (β 16) strands of the barrel. In addition, our functional assays provide the first *in vitro* evidence of the functional importance of BamA lateral gating, demonstrating that in a reconstituted system, inhibiting the lateral gating of BamA diminishes the ability of BAM to assist substrate folding. Combined, the data presented advance our mechanistic understanding of BamA function in isolation, and as part of the intact BAM complex.

P-886

Identifying the contributions of the different domains within SurA on outer member protein folding

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Outer membrane proteins (OMPs) in Gram negative bacteria, which are required for many processes, must be transported from their site of synthesis in the cytoplasm to the outer membrane, via the periplasmic space. This requires a network of molecular chaperones to prevent aggregation or premature folding. My project focuses on SurA, the primary chaperone in OMPs transport through the periplasm.

The mechanism of how SurA recognises OMPs and allows them to be inserted into the membrane by the β -barrel assembly machinery (BAM) complex, in the absence of ATP, is not fully understood. SurA is essential for the biogenesis of a number of OMPs such as LamB and its deletion results in reduced abundance of many other OMPs folded in the membrane.

I have been studying both full length SurA and SurA truncation mutants and their effects on OMP binding and delivery. The truncation mutants have one or both of the PPIase domains removed, which are not required for the chaperone activity. My results have shown that SurA binds a range non-native OMPs, with a micromolar affinity agreeing with previous findings and that removing the PPIase domains reduces that affinity. The removal of both PPIase domains also appears to reduce delivery of OmpT to the BAM complex.

P-885

Complementary cross-linking and fluorescence studies of outer membrane protein biogenesis *in vitro*

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β -barrel outer-membrane proteins (OMPs) form a large class of proteins which have a diverse range of functions in the outer membrane of Gram-negative bacteria. The β -barrel assembly machine (BAM), a multi-component protein complex, plays a major role in inserting OMPs into the outer-membrane. Being essential and ubiquitous, the BAM complex is an attractive target for novel antibiotics, but many questions regarding its mechanism and interactions remain unresolved.

To monitor the folding of model *E. coli* OMPs into lipid bilayers we are using ensemble and single-molecule fluorescence techniques to complement data collected through crosslinking and mass spectrometry. These include a new kind of assay to monitor the kinetics of OMP folding through the dequenching of extrinsic fluorophores in the presence and absence of the BAM complex and chaperones. We have produced mutant proteins with cysteine-conjugated fluorophores to perform FRET experiments between BAM subunits and model OMPs, and within OMPs themselves. Combining this with site-specific photoactivatable crosslinkers at the same cysteine positions we are able to gain information about the sites of interaction between BAM and its substrates, and the mechanisms and kinetics of β -barrel folding.

P-887

Modeling Coiled-Coil Protein Structures

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A coiled-coil protein structures consists of two or more interacting α -helical strands that together form a supercoil structure. Coiled-coil structures are critical to the function and integrity of various motor proteins, cytoskeletal filaments and extra-cellular matrix proteins. The ability to predict the propensity of two polypeptide strands to form a coiled-coil structure, at different solution conditions and external mechanical force is of special interest to a wide range of protein structure-function studies. Here we present a statistical mechanical model to predict the propensity of a given protein sequence to form a coiled-coil dimer. We calculate our model parameters based on experimental, structural and computational studies of α -helical and coiled-coil proteins. Further incorporation of this model into our previously developed α -helix tensile mechanics model enables us to predict the response of a coiled-coil structure to mechanical tension.

Posters

– 27. Protein folding and assembly –

P-888

Structural characterisation of the early events in the nucleation-condensation mechanism of foldingP. Kukic¹, Y. Pustovalova², C. Camilloni¹, S. Gianni³, D. M. Korzhnev², M. Vendruscolo¹¹Department of Chemistry, University of Cambridge, UK;²University of Connecticut Health Center, USA; ³University of Rome “La Sapienza”, Italy

A full description of the protein folding process requires the characterisation of all the states populated during the reaction. To achieve this goal we investigated the folding process of the activation domain of human procarboxypeptidase A2 (ADA2h), a protein that represents a paradigm of the nucleation-condensation mechanism of protein folding. In this mechanism, in addition to the native state, only two other states are relevant, the denatured state, where the process starts, and the transition state, where the crucial nucleation event takes place. We used a combination of relaxation dispersion NMR spectroscopy and molecular dynamics simulations, which enabled us to determine ensembles of conformations corresponding to the ground native and the low populated denatured states of ADA2h. In combination with the computational analysis of the transition state ensemble based on kinetic measurements, we found that residues that constitute the folding nucleus in the transition state most of the time form transient pairwise contacts in the denatured state in a concurrent manner, but never simultaneously. These results provide a compelling description of the nucleation-condensation mechanism from the point of view of the denatured state.

P-890

Broadband dielectric spectroscopy of bovine serum albumin in the GHz rangeE.-M. Laux¹, J. Gibbons¹, E. Ermilova¹, F. F. Bier^{1,2}, R. Hölzel¹¹Fraunhofer IZI-BB, Potsdam-Golm, Germany; ²University of Potsdam, Germany

For the investigation of the biophysical mechanisms underlying the interaction between electromagnetic fields and biological material, detailed knowledge of the electrical properties of biological substances is indispensable. Above that, by investigating these properties in response to parameters like temperature, concentration and pH, information about molecular structures and interactions of, e.g., proteins and nucleic acids can be assessed.

In the present setup, the open end of a coaxial probe is immersed into the sample solution in a standard 1.5 ml reaction tube. Amplitude and frequency of the reflected portion of an incoming radio-frequency signal are determined with the help of a vector network analyser covering the frequency range between 10 MHz and 110 GHz. From this, dielectric permittivity ϵ' and loss ϵ'' are calculated. The sample's temperature is controlled from well below 10°C to above 80°C. Dielectric properties of aqueous solutions of bovine serum albumin (BSA) are presented at protein concentrations ranging from 10 mg/ml to 800 mg/ml. Gelation is monitored, as is thermal denaturation around 60°C. The action of the denaturing chaotropic agents guanidine hydrochloride and urea is studied as well as that of the surfactant sodium dodecyl sulfate (SDS).

P-889

Protein folding via artificial macromolecular interactionS. Kwon^{1,2}, S. I. Choi³, B. L. Seong^{1,2}

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Protein folding is quite well studied, and there are many known factors that regulate protein folding. However, there are still a lot of thing that we cannot understand about protein folding. In this article we focused on effect of macromolecular interaction on protein folding, specifically steric hindrance effect and charge effect, derived from macromolecular interaction. There are many published articles saying that folding efficiency of target protein could be increased by their binding partners. These natural binding include not only steric hindrance effect and charge effect but also induced conformational change, known as induced-fit model. Thus, we used artificial macromolecular interaction to focus on steric hindrance effect and charge effect without induced conformational change. As a result, we demonstrated that artificial macromolecular interaction could raise folding efficiency of diverse protein, while molecular chaperones and natural binding partners could increase folding efficiency of the limited number of protein.

P-891

Molecular bases of D76N beta-2 microglobulin pathologic aggregation propensityT. Le Marchand¹, L. B. Andreas¹, N. Salvi², M. Bolognesi³, V. Bellotti⁴, M. Blackledge², L. Emsley⁵, C. Camilloni⁶, S. Ricagno³, G. Pintacuda¹¹CRMN, ISA, Villeurbanne, France; ²IBS, Grenoble, France;³Univ. of Milano, Italy; ⁴Univ. College London, UK;⁵EPFL, Lausanne, Switzerland; ⁶TU München, Germany

Fibrils of a newly described mutant of β -2-microglobulin (D76N β 2m), have been discovered in kindred patients suffering from gastrointestinal syndromes and autonomic neuropathy. Understanding how a single point mutation can induce such a change in pathogenicity would provide a better understanding on the mechanism of formation of fibrils.

Using a combination of ultra-fast (>60 kHz) spinning rates with 100% NH re-protonation in a perdeuterated background and high magnetic fields, we acquire sensitive ¹H-detected correlations allowing resonance assignment of both native and D76N β 2m in microcrystalline form. The resolution of 2D ¹H, ¹⁵N-HSQC spectra allowed us to investigate site-specifically the backbone dynamics of the two proteins on different timescales. In particular, ¹⁵N R₁ ρ relaxation dispersion experiments revealed that the mutation affects the ms-timescale dynamics of β 2m in very specific regions, around the mutation but also in more remote areas. The observed dynamical behavior is a result of a complex rearrangement of the network of interactions as assessed by replica-averaged meta-dynamics simulations.

Posters

– 27. Protein folding and assembly –

P-892

Single α -synuclein oligomers are hydrophobically distinct from mature fibrils

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The misfolding and aggregation of specific proteins into amyloid fibrils are hypothesized to underlie diverse neurodegenerative diseases. Alpha-synuclein (α S) protein aggregates contributing to Parkinson's disease. However, due to the complexity of aggregation process, there is still a lack of the mechanistic understanding of protein aggregation. In particular, the correlation between the morphological evolution of the aggregates and the surface hydrophobicity remains unclear. Here, we measure the surface hydrophobicity of individual protein aggregates, potentially associated with their toxicity. Using spectrally-resolved Points Accumulation for Imaging in Nanoscale Topography, we simultaneously record the localization and emission spectrum of target-bound solvatochromic dye, resulting in a hydrophobicity map of single α S aggregates at the nanoscale. By quantifying the hydrophobicity of individual aggregates at different time points, we find that oligomers are more hydrophobic and heterogeneous than mature fibrils, representing a hydrophobically non-continuum process during the α S aggregation formation. Our finding provides insight into the structural understanding of the aggregates and the correlation of hydrophobicity with toxicity of the amyloid aggregates and molecular mechanism of the disease.

P-894

Lateral and end-to-end self assembly kinetics of vimentin probed by light scattering

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The hierarchical assembly pathway of the cytoskeletal protein vimentin may be responsible for the astonishing mechanical properties of the emerging filaments, such as high flexibility and extensibility, and thus play a key role in cellular mechanics.

Intermediate filaments constitute one of the three protein filament systems in the cytoskeleton of metazoa. Together with actin filaments and microtubules they form a sophisticated composite network, which has been identified as a main player in cell mechanics.

Assembly of Vimentin from its tetrameric form can be triggered by addition of a monovalent salt. A two-step assembly mechanism, involving a lateral and a subsequent elongational step, has been established; however, the elongational step could not be followed in solution.

We present direct in situ observation and modelling of the elongation reaction of the filaments on the relevant length (60–600nm) and time scales, using time-resolved, multiangle static and dynamic light scattering. We achieve sufficient spatio-temporal resolution without the need of labelling, staining, or adsorption to substrates. The mass per unit length, hydrodynamic diameter and the end-to-end elongation rate constant of the assembling filaments are evaluated as a function of added salt.

P-893

A novel CARD assembly of the apoptosome

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Apoptosome's function is to form a wheel-like multimeric scaffold complex, via the conserved nucleotide binding oligomerization domain (NOD), in order to recruit and activate initiator caspase-9 by homotypic protein-protein interaction between the caspase recruitment domain (CARD) for triggering apoptosis, which plays an important role in development and maintaining tissue homeostasis in multicellular organisms. The *Drosophila* Dark, nematode CED-4, and human Apaf-1 apoptosomes have different CARD assemblies. Each Dark octameric apoptosome complex has eight pairs of heterodimeric CARD complexes of Dark and caspase Dronc. The CED-4 octameric apoptosome complex forms a two-layered CARD complex, whereas the heptameric Apaf-1 apoptosome complex forms an acentric CARD complex that doesn't contain seven Apaf-1 CARDs. Through rigorous efforts in the past decade, we report the first crystal structure of the CARD-CARD disk of the human Apaf-1 apoptosome. Together with the MALS and SAXS data, we show that the Apaf-1 and caspase-9 CARDs form a 3:3 complex in solution through a novel spiral DD-fold assembly. We proposed a novel assembly mechanism of how the Apaf-1 CARD and caspase-9 CARD form the CARD complex.

P-895

Structural determinants of coiled coil mechanics

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Coiled coils (CCs) are self-assembling superhelical motifs. They are naturally found in a wide range of proteins in the extracellular matrix and the cytoskeleton, where they fulfill important mechanical functions.

CCs share a characteristic heptad repeat pattern *ABCDEFG*. The hydrophobic *AD* residues drive self-assembly, while the superhelix is further stabilized by charged amino acids in the *EG* positions. The solvent-exposed residues *BCF* are hydrophilic and can be altered to tune helix stability.

With the goal of understanding the mechanistic response of CCs to an applied tensile force, we have systematically investigated the role of these 3 different classes of residues using AFM-based single molecule force spectroscopy. In detail, we have mutated the hydrophobic core residues (Ile vs. Val), altered the helical propensities of solvent exposed amino acids (Ala vs. Ser), and investigated the role of the interhelical salt bridges. Our results show that the mechanical stability of CCs depends on the stability of the hydrophobic interface and on the helicity of the CC structure. Our ultimate goal is to develop a library of CCs as mechanically calibrated building blocks for a wide range of applications: from molecular force probes to mechanoresponsive material building blocks.

Posters

– 27. Protein folding and assembly –

P-896

GTP-induced self-assembly of farnesylated hGBP1 to supramolecular complexes studied by TR-SAXS

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The human Guanylate Binding Protein 1 belongs to the family of dynamin-like proteins and is activated by addition of nucleotides which lead to protein oligomerization and to stimulated GTPase activity. [1] When the protein is expressed and purified from bacterial *E. coli* cells it lacks the posttranslational attachment of farnesyl. [2] We can show that the unmodified hGBP1 (**nf-hGBP1**) consists of monomers and dimers in nucleotide free solution whereas the natural occurring lipid-modified protein (**farn-hGBP1**) is prevented from oligomerization by farnesylation.

The GTPase mechanism of hGBP1 after addition of different nucleotides is studied using DLS, AUC and size exclusion chromatography on-line with SAXS to resolve the intermediate states during hydrolysis. Whereas the nf-hGBP1 assembles to mostly dimers and tetramers, the farnesylated protein assembles after nucleotide addition to large macromolecular structures. The polymer growth and composition is analyzed in solution using time resolved SAXS (TR-SAXS). This study shows the importance of posttranslational modifications regarding the signaling regulation and controlled growth of macromolecular complexes.

[1]: Daumke et al. *Biopolymers*. 105, 580–593 (2016).[2]: Fres et al. *J. Lipid Res.* 51, 2454–2459 (2010).

P-898

Integrative biophysical study of SGTA protein in its full-length contextS. Martinez-Lumbreras¹, E. M. Krysztofinska¹, A. Thapaliya¹, A. Spilotros², D. Matak-Vinkovic³, R. L. Isaacson¹¹Department of Chemistry, King's College London, UK;²European Molecular Biology Laboratory, Hamburg Outstation, Germany; ³Department of Chemistry, University of Cambridge, UK

The small glutamine-rich tetratricopeptide repeat protein A (SGTA) is a co-chaperone involved in diverse cellular processes such as protein quality control, tail anchored membrane protein biogenesis, steroid receptor signalling and viral lifecycles, with implications in the pathogenesis of various disease states. SGTA contains three structural domains with individual functions: an N-terminal dimerization domain that assists protein sorting pathways, a central tetratricopeptide repeat (TPR) motif that interacts with heat-shock proteins, proteasomal and hormonal receptors, and viral proteins, and a C-terminal region that binds hydrophobic substrates. A wide range of biophysical techniques has been employed to structurally characterize the different domains and investigate their interactions with binding partners, but little effort has been done in the study of the full-length version of the protein. Our current work tries to cover this gap by integrating NMR with other biophysical techniques such as SAXS, EPR or native mass spectrometry in order to characterize the domain arrangement and oligomerization state of SGTA and the mechanism of its binding to different hydrophobic substrates.

P-897

Large buffer isotope effect on protein phase behaviour and the extended law of corresponding statesN. Mahmoudi^{1,2}, S. Bucciarelli¹, L. Casal-Dujat¹, M. Jéhannin¹, C. Jud², A. Stradner¹¹Physical Chemistry, Department of Chemistry, Lund University, Sweden; ²Adolphe Merkle Institute (AMI), University of Fribourg, Switzerland

While solvent isotope effects have already provided important clues about the structural and thermal stability of disparate proteins, the effects of H/D substitution on protein phase behaviour and interactions remain to be elucidated. Here, we report the large effect of solvent isotope substitution on the phase behaviour and interactions of γ B-crystallin, a globular eye lens protein interacting via a short-range attractive interaction potential. Using a combination of scattering techniques (SAXS&SLS), we show that the liquid-liquid phase separation critical temperature T_c increases linearly from 276 K in H₂O to 292 K in D₂O. [1] Furthermore, we demonstrate that the phase boundaries and the osmotic compressibility of γ B-crystallin scale with the reduced second virial coefficient b_2 , quantifying protein–protein interactions, through the extended law of corresponding states. This thermodynamic scaling confirms the applicability of the ELCS to the equilibrium properties of colloids with short-range attractions and provides an extension of its predictive power to systems with varying hydrogen isotope content.

[1] S. Bucciarelli, N. Mahmoudi, L. Casal-Dujat, M. Jéhannin, C. Jud and A. Stradner, *The Journal of Physical Chemistry Letters*, 2016, **7**, 1610.

P-899

Framework Rigidity Optimized Dynamic Algorithm (FRODA)T. J. McManus, S. A. Wells, A. B. Walker
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We report protein structures modelled by a heuristic method, whilst still taking into account the highly important non-covalent interactions that are known to govern protein conformation. FRODA models large scale motions in constrained systems using heuristic, minimally demanding, computational methods. FRODA's validity has been widely demonstrated as a reliable companion to other, more rigorous, methods such as coarse-grained Molecular Dynamics. Constraints are applied to systems through structural analysis, which rigidifies less mobile sections. This permits larger relative motions of conjugated segments to one another through dihedral rotation of key 'hinge' like bonds. The driving forces behind these structural pathways are taken from normal mode analysis, constructing a Hessian around the assumption that changes to an equilibrated structure in a constrained system, be it lattice or protein, are derived in harmonic deviations around a starting location on the energy landscape. This work aims to carry these methods over to Fab and CDR examination and prediction in immunoglobins, whilst also improving its application in other protein structures by devising a more robust set of tools for analysing the extremely important non-covalent interactions.

Posters

– 27. Protein folding and assembly –

P-900

Exploring the conformational plasticity of tau: insights into function and dysfunctionA. M. Melo¹, J. Coraor², G. Alpha-Cobb², S. Elbaum-Garfinkle², E. Rhoades¹¹University of Pennsylvania, Philadelphia, USA; ²Yale University, New Haven, USA

Tau is an intrinsically disordered protein, whose pathological self-association is linked to several tauopathies, including Alzheimer's disease. The loss of its native interaction with microtubules is also thought to contribute to pathology. Despite intense study, structural details of the tau-tubulin complex are lacking, in part due to its highly dynamic nature and the capacity to promote tubulin polymerization. Here, we use single-molecule FRET to determine topological features of tau bound to soluble tubulin. Tau adopts an open conformation upon binding tubulin, in which the long-range contacts between both termini and the microtubule binding region (MTBR) that characterize its compact solution structure are diminished. Moreover, the individual repeats within MTBR that directly interface with tubulin expand to accommodate tubulin binding, despite a lack of extension in the overall dimensions of this region. Notably, these results suggest that the disordered nature of tau provides the significant flexibility required to allow for local changes in conformation while preserving global features. Finally, to identify domains/regions specific conformational changes in tau relevant to tauopathies, we investigate the structural consequences of tau disease mutations. The results of this work draw attention to the role of tau's conformational plasticity in function and dysfunction.

P-902

Analysis of kinetic stability model of IgGM. Nemergut¹, G. Zoldak², F. Kast³, J. V. Schaefer³, A. Plückthun³, E. Sedláč⁴¹Dept. of Biophysics, PF UPJS, Košice, Slovakia; ²Dept. of Biophysics, Institute of Molecular and Cellular Biophysics, TU München, Garching, Germany; ³Dept. of Biochemistry, University of Zurich, Zurich, Switzerland; ⁴Centre for Interdisciplinary Biosciences, PF UPJS, Košice, Slovakia

The question of stability is arguably one of the most important one in development of IgGs for pharmaceutical applications. Here, we performed detailed analysis of the kinetic stability model of thermal denaturation by methods of differential scanning calorimetry (DSC), ANS fluorescence, and absorbance spectroscopy. We show that this model correctly describes thermal denaturation of IgG in the broad protein concentrations. The correctness of the mathematical description is further supported by finding that the rate constants of the first irreversible step obtained from DSC scans very well agree with the rate constants determined by orthogonal method, kinetics of the ANS binding to IgG at various temperatures. Our analysis further show that the thermal denaturation of IgG is not affected by the aggregation of IgG and that the protein aggregation does not correlate with any of the apparent transitions. The correct values of the parameters describing individual steps in the thermal denaturation of IgG should be of important merit for: (i) predicting theoretical half-life of IgG conformational stability, (ii) ranking various IgGs according their stabilities, and (iii) models predicting the colloidal stability of IgG at different temperatures.

P-901

Conformational disorder of β – amyloid: analysis with small angle X - ray scatteringP. Moretti¹, F. Spinozzi¹, S. Morante², V. Minicozzi², M. G. Ortore¹, C. Ricci¹, G. Adriano³, B. Luigi³, B. Mariano³¹Università Politecnica delle Marche, Department of Life and Environmental Sciences - DiSVA, Italy; ²University of Rome Tor Vergata, Physics Department, Italy; ³Department of Biology, University of Padova, Italy

Intrinsically disordered proteins (IDPs) are a class of proteins that, despite the absence of a defined tertiary structure, are important in a large number of pathologies. We have studied amyloid β (A β) and α -synuclein (α -Syn) proteins, related to Alzheimer's and Parkinson disease. These peptides are prone to originate amyloid fibrils as a result of β -aggregation processes, main hallmarks of dementia [1]. An appropriate approach to study IDPs is to exploit an ensemble of conformationally disordered structures and to estimate the relative population weights according to experimental data by Bayesian methods [2]. We have developed a new method able to describe the conformational disorder of IDPs on the basis of conformational ensembles generated by random sampling and on molecular dynamics simulations coupled with the maximum entropy principle. The method is applied to the analysis of Small Angle X-ray Scattering (SAXS) data. We present the results of the method validation and its application to SAXS data recorded on A β 40 and α -Syn.

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2. R. Linding, Et Al., *J. Mol. Biol.* 342:345-353 (2004).

P-903

Structure-activity relationship of functional β -endorphin amyloid fibrils by optical SRM

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Peptide hormones are stored in an amyloid state in dense-core vesicles of secretory cells. In vitro, aggregation of hormones can be promoted by low- and high-molecular-weight compounds such as salts and glycosaminoglycans. A recent NMR spectroscopic study demonstrated that these aggregation promoters induce formation of amyloids of different atomic structure. Moreover, heparin-promoted amyloids lose their native function of reversible aggregation. Using super-resolution microscopy techniques coupled with AFM we show that heparin incorporates into β -endorphin fibrils forming an integral component and that the protein fold present in heparin-promoted amyloid seeds cannot be propagated in the absence of heparin. We also study the dynamics of two β -endorphin amyloid types and observe that salt-induced amyloids readily exchange monomers between fibrils and free monomer, while heparin-promoted amyloids do not release monomers for exchange. Observed stability of composite heparin-promoted amyloid fibrils in solution raises concerns on the biological relevance of heparin-promoted amyloid models. Given the widespread adoption of heparin as a universal aggregation promoter, the presented findings are generally relevant to the field of amyloid research.

Posters

– 27. Protein folding and assembly –

P-904

Studies toward the structure and function of the B-barrel assembly machinery in membranes

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Protein folding and proper insertion into the outer membrane of *Escherichia coli*, which is essential for physiological, pathogenic and drug resistance functions, is coordinated by the β -barrel assembly machinery. Structures of this complex, obtained in detergent micelles, were solved by a number of groups. These provide invaluable knowledge on complex assembly, and highlight its dynamic nature in these conditions. However, it is vital to study the interface of the conserved core components of this complex in a lipid environment. Our group has previously applied both solution and solid-state NMR (ssNMR) techniques to study BamA indicating that the BamA β -barrel can accommodate membrane bilayers of varying hydrophobic thicknesses. In addition we observed that POTRA domains do not display fast global motion in proteoliposomes but rather exhibit local conformational exchange, potentially involved in driving protein folding in this energy deficient environment.

In this communication we demonstrate a combined solution and ssNMR approach to study BamA and the 130 kDa subcomplex, BamAP4P5-BamCDE in liposomes. We compare our findings to published work in micelles and to recent results obtained in a (semi)-native environment.

P-906

Can we read genes?

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In principle we should be able to “read genes”, i.e. to tell the stable structures based on the gene sequence alone, but, after 60 years of active research this is still not commonly achieved. Why? In the first place since the reductionist approach is not applicable, due to the weak coupling of many interactions in each molecule. A solution to the problem depends, among other factors, on our ability to determine what sequence elements direct formation of key intramolecular contacts along the polypeptide backbone, *in situ*, in the context of the whole molecule. Our long range goal is to identify sequence elements that form the initial contacts and thus direct the pathway of fast folding. We focus our attention at the earliest formed contacts. We use various modes of FRET and fluorescence measurements, microfluidic mixing methods and perturbation mutagenesis. We hypothesize that non-local contacts along the chain are key factor in avoiding aggregation and accelerating the folding pathway. This is the “loop hypothesis” and so far we have strong evidence in support of it. Knowledge of just few non-local contacts formed early in the folding transition is key to extending the simulation of the folding to the average size proteins. New methods and results will be presented.

P-905

Near uv-visible electronic absorption originating from charged amino acids in a monomeric proteinS. Prasad¹, I. Mandal², S. Singh¹, A. Paul¹, B. Mandal¹, R. Venkatramani², R. Swaminathan¹¹Indian Institute of Technology Guwahati, India; ²Tata Institute of Fundamental Research, India

Electronic absorption spectra of proteins are characterized over 185–320 nm. Here, in a joint theoretical and experimental investigation, we report the distinctive UV-Vis absorption spectrum between 250 nm [7338 M⁻¹cm⁻¹] and 800 nm [501 M⁻¹cm⁻¹] in a synthetic 67 residue protein (α 3C) in monomeric form devoid of aromatic amino acids. Systematic control studies with high concentration non-aromatic amino acid solutions revealed significant absorption beyond 250 nm for charged amino acids which constitute over 50% of the sequence composition in α 3C. Classical atomistic molecular dynamics (MD) simulations of α 3C reveal dynamic interactions between multiple charged sidechains of Lys and Glu residues present in α 3C. TDDFT calculations on charged amino acid residues sampled from the MD trajectories of α 3C reveal that novel absorption features of α 3C may arise from charge transfer (CT) transitions involving spatially proximal Lys/Glu amino acids. The charged (NH³⁺)/(COO⁻) groups of Lys/Glu act as electronic charge acceptors/donors for photo-induced electron transfer either from/to the polypeptide backbone or to each other. Taken together, our investigation adds a novel optical 250–800 nm spectral window for probing the structure and dynamics of proteins rich in charged amino acids.

P-907

Protein-membrane interaction: insights from advanced microscopyE. Rao¹, V. Vetri^{1,2}, V. Foderà³, M. Leone^{1,2}¹Department of Physics and Chemistry, University of Palermo, Palermo, Italy; ²Aten Center, University of Palermo, Palermo, Italy; ³Section for Biologics, Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

The interaction between proteins and membranes is subject of renewed interest in biomedical and biotechnological research for its implication in many functional and dysfunctional processes and for its pharmaceutical applications. It has been shown that the interaction between amyloidogenic proteins and membranes results in mutually destructive structural perturbations. The study we present is focused on the interaction between synthetic model membranes and alpha-lactalbumin (α -La), widely studied for its biological function since it can induce apoptosis in tumor cells. Upon α -La addition to giant vesicles (GVs) samples, the system has been characterized by means of spectroscopy methods and advanced microscopy techniques. Using Raster Image Correlation Spectroscopy (RICS) and Fluorescence Lifetime Imaging Microscopy (FLIM), the interaction has been investigated at different protein:lipid ratios. Starting from the molten globule conformation, a quick insertion of α -La into the lipid bilayer takes place, with evident changes in GV morphology as well as in protein structure. The process, ruled by a combination of electrostatic and hydrophobic interactions, ends with the formation of heterogeneous structures containing both protein and lipids.

Posters

– 27. Protein folding and assembly –

P-908

SDS-induced unfolding of AfCopA, a thermophilic membrane protein

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Folding and stability of integral membrane proteins are poorly understood because of the known difficulties to find experimental conditions where reversible denaturation could be possible. In this work, we describe the equilibrium unfolding of *Archaeoglobus fulgidus* CopA, an 804 residues α -helical membrane protein involved in transporting Cu(I) throughout biological membranes. The incubation of CopA reconstituted in phospholipid/detergent mixed micelles with the detergent SDS induced a reversible decrease in fluorescence quantum yield and the loss of ATPase and phosphatase activities. Refolding of CopA from this unfolded state led to recovery of full biological activity and all the structural features characteristic of the native enzyme. Also, hydrophobic patches in CopA, mainly located in the transmembrane region, were disrupted as indicated by 1-aniline-8-naphthalenesulfonate fluorescence. Nevertheless, the unfolded state had a significant amount of residual structure. The obtained results suggest the existence of unfolding intermediates, and point out to a fine-tuning mechanism for improving protein stability.

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P-910

Interfacial self-assembly of the bacterial hydrophobin BslAM. Schor¹, G. B. Brandani¹, N. R. Stanley-Wall², C. E. MacPhee¹¹University of Edinburgh, UK; ²University of Dundee, UK

Biofilms – bacterial communities encased in a protective, extracellular, polymeric matrix – are key players in diverse processes, including chronic infections, sewage remediation and industrial biofouling. In *Bacillus subtilis* biofilms, the hydrophobin-like BslA protein forms a hydrophobic layer on the outside of the extracellular matrix, rendering the mature assembly virtually impenetrable to hydrophilic molecules including most antibiotics. In vitro, BslA self-assembles into a thin film at air/water and oil/water interfaces. This tendency to self-assemble at and stabilise interfaces is very desirable for many applications like coatings of medical implants and the stabilisation of foams and emulsions in food and personal care products.

We used a combination of experiments, modelling and all-atom molecular dynamics simulations to elucidate how the BslA proteins interact with each other when forming these films. Key amino acids stabilising different interaction interfaces have been identified using our modelling approach and were verified experimentally. This molecular level understanding of how the protein self-assembles at air-oil interfaces allows us to fine-tune the stability of the film, widening the range of potential biotechnological applications of BslA.

P-909

Interaction of the extracellular hemoglobin of *Amyntas gracilis* (HbAg) with ionic surfactantsP. S. Santiago¹, J. B. Oliveira^{1,2}, L. Ramos^{1,2}, C. Souza^{1,2}, P. G. Morgante¹¹UNESP, Registro, Brazil; ²UNESP, Araraquara, Brazil

The giant extracellular hemoglobin shows high oligomeric stability, resistance to oxidation, high cooperativity and affinity for bind oxygen. Therefore, it is very valuable for biotechnological uses as therapeutic blood substitutes. The aim of this work was to evaluate the effect of the surfactants CTAC and SDS on the oligomeric structure dissociation and oxidation by UV-vis, fluorescence and light scattering intensity (LSI). The samples were prepared as a function of CTAC and SDS concentration at pH values 5.0 and 7.0, 25 °C. At pH 5.0 and 7.0, below and above the protein isoelectric point (pI), the effects of SDS and CTAC were opposite. At pH 5.0, below the pI, occurred a lower interaction of HbAg with CTAC due to electrostatic repulsion of the surfactant positive charge with the positive charges of the protein and did not occur precipitation. However, the SDS, at pH 5.0, induced a precipitation process. The surfactant-protein aggregation was intense, generating a low LSI after centrifugation, suggesting a significant electrostatic contribution to the protein-surfactant interaction. Thus, both surfactants were able to induce oxidation followed by dissociation of HbAg, and the pH seems to influence in the type of interaction between protein-surfactant.

P-911

Volumetrically derived thermodynamic profile of interactions of urea with a native protein

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We report the first experimental characterization of the full thermodynamic profile of urea binding to a native protein. We measured the volumetric parameters of lysozyme at pH 7.0 as a function of urea at temperatures between 18 and 45 °C. Lysozyme retains its native conformation between 0 and 8 M urea over the entire range of temperatures studied. Thus, our measured volumetric properties reflect solely interactions of urea with the native protein and do not involve contributions from conformational transitions. We analyzed our data within the framework of a statistical thermodynamic analytical model in which urea-protein interactions are viewed as solvent exchange in the vicinity of the protein. The analysis produced the equilibrium constant for an elementary reaction of urea-protein binding and changes in standard state free energy, enthalpy, and entropy accompanying the binding. The thermodynamic profile of urea-protein interactions is consistent with the picture in which urea molecules, being underhydrated in the bulk, form strong, enthalpically favorable interactions with the surface protein groups. We discuss ramifications of our results for providing insights into the combined effects of urea, temperature, and pressure on the conformational preferences of proteins.

Posters

– 27. Protein folding and assembly –

P-912

Structural changes of iRFP713 protein induced by guanidine thiocyanate and guanidine hydrochlorideO. V. Stepanenko¹, O. V. Stepanenko¹, I. M. Kuznetsova¹, V. V. Verkhusha², K. K. Turoverov¹¹Institute of Cytology RAS, Russian Federation; ²Albert Einstein College of Medicine, USA

iRFP713 belongs to near-infrared (NIR) fluorescent proteins (FPs) engineered from bacterial phytochromes. NIR absorbance and fluorescence of NIR FPs make them beneficial probes for *in vivo* imaging. Spectral properties of NIR FPs are attributed to their biliverdin chromophore (BV) enzymatically derived from heme. Covalent binding of BV to NIR FPs affects their stability and folding while the place of BV attachment influences their spectral maxima. Because of the BV chromophore and an unusual knotted protein fold iRFP713 is an interesting object for folding studies. We have shown that the figure-of-eight knot in iRFP713 structure do not interfere with the protein refolding. The unfolding–refolding studies of iRFP713 denaturation induced by guanidine hydrochloride and guanidine thiocyanate (GTC) revealed a gap between the mid-point of denaturing transition, monitored by parameters of tryptophan fluorescence and chromophore fluorescence or far-UV CD. The gap was more pronounced in the presence of GTC. We corroborated these data by kinetic spectroscopic and steady-state gel-filtration studies. We have revealed two intermediate states and characterized them as native-like monomeric and aggregated states. This work was supported by the MCB Program of RAS and RFBP grant (16-04-01515).

P-914

Structure of coumarin derivatives affects amyloid aggregation of lysozymeK. Ulicna^{1,2}, Z. Bednarikova², W.-T. Hsu³, S.-S.-S. Wang³, S. Hamulakova⁴, M. Holztragerova¹, Z. Gazova²

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We have focused on studying the anti-amyloid properties of 10 coumarin derivatives divided to 3 structurally different groups based on the type of the linker connecting the tacrine, acridine and coumarin functional groups. Using the *in vitro* and *in silico* approaches we have examined the effect of studied compounds on the formation/reversion of amyloid aggregation of HEWL. Our results indicate that the anti-amyloid activities of studied derivatives depend on their structure. The most efficient inhibitors were tacrine/coumarin derivatives with alkylenediamine linkers. They inhibited fibrillization of lysozyme and reduced the formation of amyloid fibrils at stoichiometric concentrations. *In vitro* cell culture experiments showed that cytotoxicity of all coumarin derivatives is very low. The obtained results can be helpful for the design and development of new therapeutics for amyloid-related diseases.

Acknowledgement

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P-913

Investigating the unfolding pathway of sensory rhodopsin II

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Membrane protein misfolding has strong clinical implications (e.g. misfolding of rhodopsin in Retinitis pigmentosa), but studies on their folding and unfolding mechanisms are sparse. Sodium dodecyl sulphate (SDS) is commonly used for studying the unfolding of membrane proteins, with the advantage of preserving the proteins in a micellar environment. Type I and Type II rhodopsins are seven transmembrane helical proteins that share structural similarities to G protein-coupled receptors. Previous studies on SDS-denatured bacteriorhodopsin and bovine rhodopsin suggest different unfolding pathways despite similarities in the native folded structures. SDS-mediated unfolding of sensory rhodopsin II (pSRII), an archaeal Type I rhodopsin, is studied with the aim of elucidating molecular properties which lead to mechanistic differences in unfolding. Biophysical studies show that SDS-denatured pSRII remains alpha-helical, despite losing native retinal-protein contacts and retinal covalent linkage. Solution NMR studies have led to insights on sequential changes in functional contacts and conformational mobility of pSRII along its unfolding pathway. Overall, pSRII unfolding is characterised by loss of inter-helical contacts and little loss in secondary structure, similar to bacteriorhodopsin.

P-915

Great interaction: Binding incorrect protein partners to learn about recognition and functionL. Vamparys¹, A. Carbone², S. Sacquin-Mora¹

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Protein interactions play a key part in most biological processes and understanding their mechanism is a fundamental problem leading to numerous practical applications since proteins represent a major class of therapeutic targets. Docking simulations between two proteins known to interact can be a useful tool for the prediction of likely binding patches on the surface. From the analysis of the protein interfaces generated by a massive cross-docking experiment using over 150 proteins, where all possible protein pairs, and not only experimental ones, have been docked together, we show that it is possible to predict a protein's binding site without having any prior knowledge regarding its potential interaction partners. Furthermore, a new clustering analysis performed on the binding patches scattered on the protein surface show that their distribution and growth will depend on the protein's functional group. In several cases, the binding-site predictions resulting from the cross-docking simulations will lead to the identification of an alternate interface, which corresponds to the interaction with a biomolecular partner that is not included in the original benchmark. **Keywords:** Protein-Protein interaction, docking, coarse-grain model

Ref. Vamparys et al., *Proteins* **2016**, 84, 1408.

Posters**– 27. Protein folding and assembly –****P-916****Probing the conformational dynamics of proteins with high multiplexed magnetic tweezers**

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Magnetic Tweezers (MT) are a single molecule technique that enables the application of both forces and torques to biological macromolecules such as DNA or proteins. The molecules of interest are attached with one end to super-paramagnetic beads, while their opposite ends are attached to the bottom surface of a flowcell. Magnets, placed above the flowcell, exert magnetic fields such that a constant force is applied to the molecules, without the need for feedback. Here, we present force clamp measurements of proteins. We are exploring the general requirements for protein unfolding measurements in MT, such as passivation of the surface, attachment strategies to bind the proteins covalent to the surface as well as to the beads, and the right concentration of functional proteins bound to the surface. In our proof-of-concept measurements, we are investigating Green-Fluorescent-Protein (GFP) unfolding events to use as a fingerprint for future measurements of force clamp force-activation and unfolding of functional proteins.

P-917**NMR measurements of dynamics and protein-ribosome interactions in ribosome–nascent chain complexes**

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NMR measurements of nuclear spin relaxation provide extraordinarily powerful probes of polypeptide dynamics across a range of timescales. In particular, measurements of cross-correlated relaxation processes provide a valuable description of ‘pure’ dynamics within a spin system, free from contributions due to chemical exchange or interactions with external spins. However, such measurements have typically been associated with low experimental sensitivity. Here, we report on the development of sensitivity-optimized pulse sequences for the measurement of cross-correlated relaxation in amide and methyl spin systems, and also describe the application of optimal design theory to implement ‘on-the-fly’ adaptive sampling schemes, calculated in real time during acquisition, that maximize the accuracy of the measured rate constants. We describe the application of these methods to quantify the ribosome surface interaction of folded and unfolded states of translationally-arrested ribosome–nascent chain complexes of the FLN5 filamin domain (Cabrita *et al.* (2016) *Nat. Struct. Mol. Biol.*).

P-918**In-situ study on conformational change of PMP22-TM4 in a SERS-active microchannel**

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Peripheral myelin protein 22 (PMP22) is known to play important roles in regulating Schwann cell proliferation and in myelin formation and maintenance. But mutants of PMP 22 are prone to be misfolded and result in different peripheral neuropathies. In this work, we in-situ studied the conformational changes of the fourth transmembrane helix of PMP 22 (PMP22-TM4) and its mutant G150D in a SERS-active microchannel under different environmental conditions.

By flowing PMP22-TM4, both wild-type (WT) and G150D, into a SERS-active microchannel, the SERS signals were collected, meanwhile, PMP22-TM4 was adsorbed in the microchannel. Then, various concentrations of denaturant (GuHCl) were flowed into the channel. Finally, buffer were pumped into the channel and washed out the denaturant. SERS signals of PMP22-TM4 in different concentrations of GuHCl clearly showed the differences of WT and G150D in processes of unfolding and refolding. G150D is much more sensitive to the denaturant than WT and its SERS spectra are almost same at 3M and 6M GuHCl. Besides, SERS signals revealed the different adsorb modes of WT and G150D on SERS substrate. The native WT “stands” on the substrate, but the refolding WT has to “lie” on the substrate; while both native and refolding G150D “lie” on the substrate.

Posters

– 28. Morphogenesis and development –

P-919 (O-168)

Epithelium adaptation to external curvature *in vitro*

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Deformation of flat epithelia into a given shape is specific to each organ and its function in the organism. For instance, during the gut formation, an initially smooth gut tube is formed and then intestinal villification can take origin by muscle constriction (Shyer et al., Science, 2013). Once the villi shape is established, it is maintained throughout of life. Therefore, gut cells have to function in regions of different curvature. Despite of the growing evidence of the interplay between external forces, mechanotransduction and organ morphology, little is known about cell adaptation to external geometrical constraints. For this study, we have developed two complementary techniques to control epithelium curvature and to investigate its possible role in cell growth and organisation. In the first case we induce an initially flat epithelium on PDMS substrates to deform into a given curvature; in the second case we investigate epithelial cell growth encapsulated in alginate tubes. These systems have the advantage to provide simple tools to control the physical cell environment and to isolate the effects of its properties on cell growth. In particular, our researches focus on quantitative studies of epithelial monolayer adaptation, e.g. in terms of cell shape and proliferation.

P-921 (O-166)

Decoding temporal interpretation of the morphogen Bicoid in the early *Drosophila* embryo

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Morphogen gradients provide essential spatial information during development. Not only the local concentration but also duration of morphogen exposure is critical for correct cell fate decisions. Yet, how and when cells temporally integrate signals from a morphogen remains unclear. Here, we use optogenetic manipulation to switch off Bicoid-dependent transcription in the early *Drosophila* embryo with high temporal resolution, allowing time-specific and reversible manipulation of morphogen signalling. We find that Bicoid transcriptional activity is dispensable for embryonic viability in the first hour after fertilization, but persistently required throughout the rest of the blastoderm stage. Short interruptions of Bicoid activity alter the most anterior cell fate decisions, while prolonged inactivation expands patterning defects from anterior to posterior. Such anterior susceptibility correlates with high reliance of anterior gap gene expression on Bicoid. Therefore, cell fates exposed to higher Bicoid concentration require input for longer duration, demonstrating a previously unknown aspect of morphogen decoding.

P-920 (O-167)

The physical basis of coordinated tissue spreading in zebrafish gastrulation

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Embryo morphogenesis relies on highly coordinated movements of different tissues. Yet, remarkably little is known about how tissues coordinate their movements to shape the embryo. Tissue coordination becomes first apparent during one of the main stages of vertebrate development, namely gastrulation. In zebrafish embryos, the onset of gastrulation is characterised by the spreading of a tissue, the blastoderm, over a yolk sac and this process is commonly thought to be mediated by radial cell intercalations. These movements simultaneously narrow the tissue along its height (radial extent) and expand it along its plane. Yet, whether radial cell intercalations drive tissue spreading or represent the response of the tissue to exogenous spreading forces remains unclear. In this talk, we use a combination of active gel theory and experiments (by Dr. Hitoshi Morita) to dissect the fundamental force-generating processes underlying the initial spreading of the blastoderm over the yolk cell at early zebrafish gastrulation. Unexpectedly, we found that active radial cell intercalations are dispensable for blastoderm spreading *per se* and that, instead, this process is driven by epithelial surface cells autonomously reducing their surface tension and thus actively expanding.

P-922

A computational study of the effect of plasma skimming on vascular development

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In recent work, we have pioneered the developed computational models of retinal blood flow for the study of vascular development (Bernabeu et al., J. R. Soc. Interface, 2014) and diabetic retinopathy (Lu et al., Biomed. Opt. Express, 2016). In these works, blood was modelled as a shear-thinning homogeneous fluid rather than a particle suspension. This simplification, often required for computational tractability reasons, is appropriate for the study of haemodynamics in vessels with diameters larger than a few hundred micrometres. However, it fails to capture important rheological properties when applied to the simulation of blood flow in capillaries. In the current work, we successfully implemented a model of deformable particles into the open source HemeLB Computational Fluid Dynamics solver (<http://ccs.chem.ucl.ac.uk/hemelb>). Example simulations in developmental vascular networks are discussed and the differences in the wall shear stress (WSS) predicted by a homogeneous shear-thinning rheology model and a particle suspension rheology model are studied. Based on our results, we hypothesise that plasma skimming enhances the WSS gradients known to modulate developmental vascular remodelling (Franco et al., PLoS Biol., 2015).

Posters

– 28. Morphogenesis and development –

P-923

Skeleton construction of sponges: self-building up by dynamic transport and assembly of spicules

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Determining the mechanisms of how a skeleton forms is one of the important issues for understanding how the various forms of animal bodies are formed. We revealed a fundamentally novel mechanism of skeleton construction using a demosponge, *Ephydatia fluviatilis*. Sponges' skeleton is self-organized as a result of the iterations of sequential physical reactions of player cells ("in situation A, do B"). We succeeded in live imaging how fine spicules (glass needle-like structures) are assembled to construct a skeleton (basically a large pole and beam structure). Unexpectedly, spicules are dynamically "carried" by newly discovered transport cells, and then the spicules "pierce" outer epithelium to stick out from the sponge body, the front end of the spicule "raised up", then, and then the basal end is "cemented" by embedding in the thickened collagenous matrix under the basal epithelia or connected to the spicule-constructed skeleton to elongate a spiculous pole (spicule tract) (Nakayama et al. 2015 *Cur.Biol.*). We recently attempted to determine how physical forces from the environment (such as water flow) influence the skeleton construction of sponges using our unique experimental approaches. The mechanisms that produce the phenotypic plasticity of sponges will be discussed.

P-925

Quantitative characterization of electrotropism in Arabidopsis roots

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Plant roots are exposed to a wide range of physical and chemical stimuli in soil. In these conditions, an important response is the reorientation of growth in relation to perceived signals (tropism), such as gravity (gravitotropism), light (phototropism), pressure, or touch (thigmotropism), water (hydrotropism), oxygen (oxytropism) and other chemical gradients (chemotropism).

We are studying the ability of roots to sense and align with local electric fields (electrotropism). These are weak and often transient electrostatic fields that naturally occur in soil when physical (e.g. water flow) or biological (e.g. micro-organisms) conditions generate non-uniform distribution of charges, such as ions.

We will present unpublished quantitative phenomenology of root electrotropism reproduced in controlled laboratory conditions, using the plant model system *Arabidopsis thaliana*. We use time-lapse microscopy to quantitate the morphological response at both the organ and cellular scales, and to study the dynamics of biochemical and transcriptional spatial patterns *in situ*.

We will discuss possible cellular and molecular mechanisms involved in root electrotropism, and propose future directions and potential applications for this exciting field of research.

P-924

Partner search strategy and mechanisms of polarization during fission yeast mating

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Nitrogen starvation triggers sexual differentiation and mating in the model organism fission yeast. The two sexual cell types P and M secrete specific P- and M-factor pheromones, respectively, which bind cognate receptors on the partner cell surface to promote the growth of a mating projection. The location of the projection is established after the small GTPase Cdc42, together with its GEF Scd1 and the scaffold protein Scd2, forms an exploratory patch that samples the cell periphery. We used a combination of computational modeling and experiments to model the role of the exploratory Cdc42-GTP zone that appears and disappears along the cortex and stabilizes in response to secreted pheromone. We found that efficient pair formation occurs through the combination of local pheromone release, short pheromone decay length, and local pheromone sensing. Interestingly, deletion of Gap1, the GTPase activating protein for Ras1, an upstream activator of Cdc42, leads to patch stabilization at lower pheromone concentrations. We propose that negative regulation of Ras1 GTPase by Gap1 promotes the exploratory mechanism. Upon cell pair formation, the Ras activator-inhibitor dynamic system along the cell membrane undergoes a transition from exploratory to stable state in response to opposite mating type pheromone.

P-926

The Regulation of Spindle Positioning by p37

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Spindle orientation regulates the axis of division and therefore contributes to tissue morphogenesis and homeostasis. Our lab has shown that p37, a cofactor of a p97 AAA ATPase, is involved in spindle orientation. However, the mechanisms by which p37 controls this process were unknown.

Spindle orientation is regulated by cortical forces, that are generated by the dynein-motor protein. In metaphase, the membrane-anchored G α i/LGN complex recruits NuMa, which, in turn recruits dynein to the cortex. We found that p37 depletion results in high cortical NuMa levels. Partially depleting NuMa in p37 lacking cells rescues the spindle orientation defect, indicating that p37 regulates spindle orientation by limiting cortical NuMa level. Moreover, cortical NuMa is still present in cells lacking both G α i and p37, indicating that p37 negatively regulates an alternative NuMa recruitment pathway.

Cortical NuMa levels are also regulated by phosphorylation. We found that co-depleting p37 and the phosphatase PP1 or its regulatory subunit RepoMan suppresses the high cortical NuMa levels and rescues the spindle orientation defect. Therefore, our data identifies a new regulator of NuMa cortical recruitment, PP1/RepoMan and shows that p37 restrains this pathway in metaphase cells.

Posters**– 28. Morphogenesis and development –****P-927****Epilepsy-associated gene Pk regulates neurite outgrowth through stabilizing neuro-glia interaction**T. Liu, X. Li, Y. Li

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Epilepsy is a neurological disorder characterized by recurrent seizures. Although a number of genetic mutations have been associated with this disease, the pathogenesis mechanisms remain complex. Prickle, known as a core component protein of PCP pathway, is closely associated with epilepsy; however, its function in the neuron system yet wait elucidation. There are three proteins in *Drosophila*, Pk, PkM, and Spiny-legs (Sple), derived from a single gene through alternative splicing. In the mutant flies, we found abnormal neurite outgrowth, which was resulted from the deficiency in neuro-glia interaction. Moreover, these defects were due to the failure of membrane localization of adhesion molecules. We further found that Pk and Sple coordinately regulate the molecular machinery in the control of neuron-glia interaction. Therefore, our findings suggested that demyelination might be a cause in Pk-associated seizure formation.

Posters

– 29. Optogenetics and neural systems –

P-928 (O-172)

A novel neurophotonic approach to study neural networks *in vitro*

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Optogenetics is an emerging field that benefits from the synergy of optical and genetic techniques [1]. It allows non-invasive activation or inhibition of specific neurons using only light and enables repetitive interrogations of the same cells. It has been combined with purely optical readout of neural activity (using fluorescent voltage reporters) in a technique called Optical Electrophysiology [2] but this requires extreme microscopy which limits its use in pre-clinical and drug screening applications. To overcome these limitations, we have recently developed advanced protocols in which calcium imaging can be used to quantify network dynamics *in vitro* using a genetically encoded calcium reporter (manuscript in preparation). This is a novel quantitative use of neurophotonic to potentially understand human cognition, investigate how diseased neurons communicate thus enabling functional *in vitro* high throughput screening and testing of dementia drug candidates.

1. Boyden, E.S., et al., *Millisecond-timescale, genetically targeted optical control of neural activity*. Nat Neurosci, 2005. 8(9): p. 1263-1268.

2. Hochbaum, D.R., et al., *All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins*. Nat Methods, 2014. 11(8): p. 825-33.

P-930 (O-174)

Orchestrating cells on a chip employing standing surface acoustic waves towards neural networksM. S. Brugger¹, S. Grunden², L. Theogarajan²,A. Wixforth^{1,3}, C. Westerhausen^{1,3}

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We propose the implementation of a new lab-on-a-chip based system for the controlled growth and formation of complex neural networks on a semiconductor chip. By combining microfluidic techniques with surface acoustic waves (SAW), we can create and stimulate simple life-on-a-chip systems. For this purpose, we constructed a chip consisting of a piezoelectric LiNbO₃ substrate and four interdigital transducers for the excitation of SAW to from standing waves with according nodes and antinodes in a checkerboard pattern. The anticipated formation of the pressure node lattice has been visualized using atomic force microscopy. By adding a PDMS-microchannel, this equidistant and regular patterning lattice allows us to simultaneously control the position of objects in a liquid environment in space and time. The possibility and accuracy to pattern cell-sized single objects on these chips were validated by patterning small beads of different sizes. Ensuring the conditions for cell growth, we successfully demonstrate single cell alignment, their adhesion and growth within the well-defined pressure nodes on the chip. Finally, we verified the biocompatibility of SAW for primary neural cells. This gives us confidence that it will allow us to apply our technique to single neurons in the future.

P-929 (O-173)

Signaling states of short LOV proteins and their implications for construction of optogenetic toolsR. Batra-Safferling¹, J. Granzin¹, V. Arinkin¹, K. Röllén¹, U. Krauss², K.-E. Jaeger², D. Willbold¹

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Unique features of Light-Oxygen-Voltage (LOV) proteins like relatively small size (~12–19 kDa), inherent modularity, highly-tunable photocycle and oxygen-independent fluorescence have lately been exploited for the generation of optogenetic tools.

Here, we will provide insights of the light-activated signaling mechanism in a short LOV protein PpSB1-LOV from a comparison of crystal structures obtained in the dark- and fully light-adapted states. Major structural differences involve a ~11 Å movement of the C terminus in helix Jα, disruption of the dimer interface, and a ~29° rotation of chain-B relative to chain-A in the dark state. The activation mechanism supports a rotary switch mechanism and provides insights into the signal propagation mechanism in naturally existing and artificial LOV-based, two-component systems.

Second, we will present the first apo-state crystal structure of a PpSB1-LOV homologue- W619_L1 (89% sequence identity). The apo protein binds both natural and structurally modified flavin chromophores, revealing remarkably different photophysical and photochemical properties. These results imply application of these selective variants as novel optical tools as they offer advantages such as no adduct state formation, and a broader choice of wavelengths for *in vitro* studies.

P-931

Activation and silencing of specific neurons via light and temperature using the MEA technologyE. Dragicevic¹, C. Weichbrodt¹, D. Millard², I. Clements², A. Nicolini², S. Chvatal², M. Clements², J. Ross², M. George¹, N. Fertig¹

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Multiwell microelectrode array (MEA) systems provide simultaneous measurements of extracellular electrophysiological activity of excitable cells over long periods of time. Each electrode is capable of capturing extracellular action potentials of excitable cells in ultrahigh resolution (millisecond events with microvolt amplitudes), while multiple recording sites within each well allow population network activity measurements. The MEA technology has been used for neuronal network research for several years and offers unbiased, label free, non-invasive recordings of neuronal cell functions, in a regulated physiological environment. Using optogenetic stimulation and precise temperature control, we show that the activity of specific neuron populations can be controlled. With this, the novel solutions for specific cell stimulation (pacing) or silencing using multiwell light delivery add-ons for optogenetics further excels MEA-based disease modeling and drug discovery. Through even illumination of the wells and lack of induced artifacts, optogenetic stimulation exhibits improved reliability across wells, as compared to electrical stimulation. Here, we demonstrate the control of neuronal activity by temperature, electrical and optogenetic stimulation, using different iPSC-derived neuronal cell types.

Posters

– 29. Optogenetics and neural systems –

P-932

Non-invasive measurement of force exerted by multiple motor proteins during the axonal transportK. Hayashi¹, Y. Tsuchizawa^{2,3}, M. Iwaki³, Y. Okada^{3,4}¹Graduate School of Engineering, Tohoku University, Japan; ²Graduate School of Frontier Bioscience, Osaka University, Japan; ³Quantitative Biology Center, RIKEN, Japan; ⁴Graduate School of Science, The University of Tokyo, Japan

Here we report a new non-invasive measurement of the force exerted in the living neurons. The axonal transport of membrane vesicles in neurons were analyzed, because very basic properties such as the force exerted by motor proteins during transport is still undetermined. We have analyzed the fluctuation of the displacement of the axonally transported endosomes, which enabled the estimation of the drag force exerted on the endosomes during transport. Its distribution exhibited several distinct peaks at multiples of a unit force. This result, not only suggests the existence of a unit for force production, but also demonstrates that a single cargo vesicle is often conveyed by several force producing units.

P-934

Characterization of mitochondrial ferritin-overexpression mice

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Mitochondrial ferritin (FtMt) is a H-ferritin-like protein which localizes in the mitochondrial. More and more studies have shown that this protein can protect mitochondrial from iron-induced oxidative damage and it's overexpression in cultured cells protected against oxidative damage. To investigate the *in vivo* role of FtMt, we established *Ftmt* overexpression mouse by using pronucleus microinjection and examined the characterization of this mouse. We confirmed the mRNA and protein expression level of *Ftmt* in transgene mice were remarkably increased. Our data show that there were no significant differences between these two mice on body weight and organ coefficient. We also measured serum, spleen, heart, kidney, testis, liver and brain iron concentrations, liver hepcidin expression and red blood cell parameters. There were no significant differences. In conclusion, these results suggest that *Ftmt* overexpression mice didn't have over phenotype and we predict the effect of *Ftmt* overexpression can be represented under stress conditions.

P-933

A unique choanoflagellate enzyme rhodopsin with cyclic nucleotide phosphodiesterase activityS. Tsunoda^{1,2}, K. Yoshida², L. Brown³, H. Landori²¹PREST/JST, Japan; ²Nagoya Institute of Technology, Japan; ³University of Guelph, Canada

We report on an unusual microbial rhodopsin which may be able to meet the demand. It is found in the choanoflagellate *Salpingoeca rosetta* and contains a C-terminal cyclic nucleotide phosphodiesterase (PDE) domain. We examined enzymatic activity of the protein (named Rh-PDE) both in HEK293 membranes and whole cells. Even though Rh-PDE was constitutively active in the dark, illumination increased its hydrolytic activity 1.4-fold toward cGMP and 1.6-fold toward cAMP, as measured in isolated crude membranes. Purified full-length Rh-PDE displayed maximal light absorption at 492 nm and formed the M intermediate with the deprotonated Schiff base upon illumination. The M state decayed to the parent spectral state in 7 seconds, producing long-lasting activation of the enzyme domain with increased activity. We discuss possible mechanism of the Rh-PDE activation by light. Furthermore, Rh-PDE decreased cAMP concentration in HEK293 cells in a light-dependent manner and could do so repeatedly without losing activity. Thus, Rh-PDE may hold promise as a potential optogenetic tool for light control of intracellular cyclic nucleotides, for example, to study cyclic nucleotide-associated signal transduction cascades.

P-935

Hypobaric hypoxia regulates brain iron homeostasis in rats

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Disruption of iron homeostasis in brain has been found to be closely involved in several neurodegenerative diseases. Recent studies have reported that appropriate intermittent hypobaric hypoxia played a protective role in brain injury caused by acute hypoxia. However, the mechanisms of this protective effect have not been fully understood. In this study, Sprague-Dawley rat models were developed by hypobaric hypoxia treatment in an altitude chamber, and the iron level and iron related protein levels were determined in rat brain after four weeks of treatment. We found that the iron levels significantly decreased in the cortex and hippocampus of rat brain as compared to that of the control rats without hypobaric hypoxia treatment. The expression levels of iron storage protein L-ferritin and iron transport proteins, including transferrin receptor-1 (TfR1), divalent metal transporter 1 (DMT1) and ferroportin1 (FPN1), were also altered. Further studies found that the iron regulatory protein 2 (IRP2) played a dominant regulatory role in the changes of iron hemostasis, whereas iron regulatory protein 1 (IRP1) mainly acted as cis-aconitase. These results, for the first time, showed the alteration of iron metabolism during hypobaric hypoxia in rat models, which link the potential neuroprotective role of hypobaric hypoxia treatment to the decreased iron level in brain. This may provide insight into the treatment of iron-overloaded neurodegenerative diseases.

Posters**– 29. Optogenetics and neural systems –****P-936****Towards all-optical functional imaging and optogenetic manipulation of the insect brain**

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We present our efforts to develop and optimize an optical setup combining multiphoton microscopy with local optogenetic stimulation, in order to measure and modulate neuronal activity in the insect brain. This is an interesting model in neurobiology, thanks to its small size, easy structure but rich performances. We use two-photon microscopy to image morphology and functions of the insect olfactory system to study various aspects of odour coding.

We present first steps to add optogenetics to our experimental toolbox. Optical activation of opsins, expressed in selective neurons and addressable via an additional scannable laser source, will allow to modulate neuronal activity with high selectivity and precision both in the temporal and spatial domain. This will permit to study the role of single functional units in local neural networks, by stimulating specific nodes while recording the response in others, and to measure the network's connectome also in the resting state, independently of global stimuli.

Functional response studies will go beyond mapping out average activity, by analyzing also temporal features, e.g. via space-by-time factorization of the activity patterns. This will allow to study the role of the temporal dimension in stimulus coding in the somatosensory system.

P-937**Arginine vasopressin reduces apoptosis and ameliorates spatial learning impairments in global cerebral ischemia model rats**

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Ischemia and hypoxia are the main causes of decline in spatial learning and memory, which is involved in neuron apoptosis. Although arginine vasopressin (AVP) has been strongly involved in learning and memory, and the effects of AVP on ischemia rat model remain unclear. In our study, we aimed to investigate whether there was neuroprotective effects of AVP on ischemia rat model, and the role of apoptosis in this process. The global cerebral ischemia rat model was established by bilateral carotid arteries occlusion, and the secretion of AVP was induced by injection of hypertonic saline (5.3 %) i.p.. Results showed that hypertonic saline effectively elevated the expression of AVP and decreased the apoptosis level compared to those of ischemia rats. Moreover, the synaptic plasticity of CA3-CA1, as well as the performance in MWM were enhanced by the injection of hypertonic saline in ischemia rats. These findings imply that AVP plays a helpful role for the treatment of cognitive impairments in ischemia rats.

Keywords: Ischemia model rats, Arginine vasopressin, Apoptosis, Learning and memory

Posters

– 30. Ionic liquids meet biomolecules –

P-938

Water modulates the dynamics and stability of hydrated ionic liquids near DNA

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The thermodynamics effect of DNA stability in the presence of ionic liquids (ILs) is well studied. In this talk we will discuss how the dynamics of ionic liquid effects the DNA structure. Using molecular dynamics simulation, we calculated the mean residence time (MRT) of the cations of five different ILs in the grooves and around phosphate groups of AT and CG rich DNA segments. We find the residence time of different cations next to CG rich DNA to be much higher compared to that next to AT rich DNA, with a negligible difference with the variation of anions. The interaction energy between cations and DNA, however, shows exactly the opposite trend; it is much lower (indicating a stronger interaction) for AT than for CG. Investigation of DNA parameters reveals an insignificant difference for the DNA sequences under consideration. Analysis of water behavior provides a rationale for the long MRTs of cations; water molecules have been found to be denser and to possess higher MRT when next to CG-rich DNA, thus resulting in a crowded environment. Our results indicate that the dynamics influence the binding of ILs to different DNA sequences, possibly by modulating the entropy of the binding process.

P-940 (O-180)

Biophysical and biological activities of imidazolium-based lipid analoguesD. Wang¹, A. Rühling², P. Drücker¹, D. Grill³, F. Glorius², H.-J. Galla¹

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Tailor-made ionic liquids based on imidazolium salts have attracted our attention due to their extraordinary properties and versatile functionality. Here we report the intriguing ability of newly synthesized 1,3-dialkylimidazolium compounds to interact with and to stabilize lipid membranes. The introduction of alkyl chains at the imidazolium backbone in the 4,5-position of N-heterocyclic carbene salts leads to a high structural similarity to the hydrophobic part of natural lipids. Their interaction with cellular membranes resulted in remarkable properties such as enhanced cytotoxicity and antitumor activity, which was interpreted by surfactant monolayer models in vitro. Membrane hydration properties and domain fluidization were analyzed by fluorescence in lipid bilayers resembling living cells. Membrane binding and insertion was analyzed via a quartz crystal microbalance and confocal laser scanning microscopy. It is shown that short-chain 4,5-dialkylimidazolium salts with a bulky head group are able to disintegrate membranes. Long-chain imidazolium salts form bilayer membrane vesicles spontaneously and autonomously without the addition of other lipids.

P-939 (O-179)

Probing interactions of cellulose, lignin and ionic liquids towards enabling sustainability

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Carbon-rich fossil fuels provide majority of the energy consumed in the world. The high-energy content of liquid hydrocarbon fuels makes them the preferred energy source for all modes of transportation. However, non-renewable, fossil fuel based society is not sustainable. This has spurred research into alternative, non-fossil energy sources and among the options, biomass has the potential to provide a high-energy-content transportation fuel. Plant-derived biomass contains biological macromolecules like cellulose, hemicellulose and lignin. Ionic liquids (ILs) based biomass conversion technologies have recently gain a great deal of attention as a green option that could enable carbon-efficient conversion. However, the development of cost-effective processes to transform cellulose and lignin in biomass into fuels is hampered by poor understanding of fundamental biophysical processes at play and necessitate application of advanced experimental and theoretical approaches to gain a better understanding of these interactions. I will discuss both computational and experimental approaches we have been using to gain a better understanding of ILs interactions with cellulose and lignin and how our effort is enabling a whole new class of renewable ILs for bio-based economy and sustainability.

P-941 (O-181)

Interaction of imidazolium-based ionic liquids with soft supported lipid membraneG. Bhattacharya¹, R. P. Giri², H. Saxena¹, V. V. Agrawal³, A. Gupta¹, M. K. Mukhopadhyay², S. K. Ghosh¹

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Ionic liquids (ILs) are known to show antimicrobial activity and are observed to be toxic to some microorganisms. To understand the molecular mechanism of these activities, the interactions of imidazolium-based ILs with model biomembranes have been investigated. The pressure area- isotherm of self-assembled monolayer formed by cell membrane mimicking lipid molecules is found to be altered due to interaction with ILs. As a result, the in-plane elasticity of the monolayer is reduced. The self-assembled structure of lipid bilayer on a polymer support is perturbed as evident from x-ray reflectivity (XRR) studies. Due to the IL-membrane interaction, the bilayer thickness reduces considerably and the corresponding electron density of the layer is increased (1). The nature of the interaction not only depends on the hydrophobicity of ILs but also on the electrostatics of the system. This study provides a molecular description of IL-membrane interaction. References

(1) G. Bhattacharya, R.P Giri, H. Saxena, V. V Agrawal, A. Gupta, M.K Mukhopadhyay, S.K Ghosh, *Langmuir* **33**, 1295 (2017).

Posters

– 30. Ionic liquids meet biomolecules –

P-942

Protein-polymer surfactant nanoconjugates for biocatalysis in anhydrous ionic liquids

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Enzymes can perform many industrially relevant reactions with high specificity and efficiency. However, enzymes often have very low solubilities and stabilities in the nonaqueous environments required industrially. Consequently, there is a need to develop new biotechnologies that improve solubility and stability of biocatalysts in nonaqueous media.

Surface modification of proteins, to yield protein-polymer surfactant nanoconjugates, has been demonstrated as a robust method for synthesizing protein-rich biofluids. Using a variety of spectroscopic and scattering techniques, these novel biomaterials have been shown to allow for extreme enzyme thermal stability, and enhanced enzyme activity.

Recently, we showed that protein-polymer surfactant nanoconjugates are soluble in anhydrous ionic liquids. Furthermore, the solubilized protein had preserved structure and displayed improved thermal stability as compared to aqueous solutions. Here, we show recent results involving the enzyme β -glucosidase. Particularly, we demonstrate that the enzyme has significantly improved activity in ionic liquids at 120 °C. As a result, this nascent technology could provide a platform for biocatalysis in industrially relevant nonaqueous solvent systems.

P-944

Can an ammonium-based room temperature ionic liquid counteract the urea-induced denaturation?

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The folding/unfolding equilibrium of proteins in aqueous medium can be altered by adding small organic molecules generally termed as co-solvents. Denaturants such as urea are instrumental in the unfolding of proteins while protecting osmolytes favour the folded ensemble. Recently, room temperature ionic liquids (ILs) have been shown to counteract the deleterious effect of urea on proteins. In this paper, using atomistic molecular dynamics we show that a ternary mixture containing a particular ammonium-based IL, triethylammonium acetate (TEAA), and urea (in 1 : 5 molar ratio) helps a small 15-residue S-peptide analogue regain most of its native structure, whereas a binary aqueous mixture containing a large amount of urea alone completely distorts it. Our simulations show that the denaturant urea directly interacts with the peptide backbone in the binary mixture while for the ternary mixture both urea as well as the IL are preferentially excluded from the peptide surface.

P-943

Stability and aggregation of lysozyme in water-miscible ionic liquids

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Ionic liquids (ILs) are frequently used as solvents in many various fields. The growing interest in ILs is based on their unique physico-chemical properties (polarity, viscosity, thermal stability, conductivity, low volatility). The desired properties of ILs can be achieved by appropriate combination of cations and anions or additional solvents.

We have studied the effect of water-miscible imidazolium- and pyrrolidinium-based ILs in wide ILs concentration range on thermal stability and aggregation of lysozyme. The aim of this work is to characterize the effect of different chaotropic cations and their combinations with kosmotropic and chaotropic anions on stability of lysozyme. We are focused on finding proper conditions inducing formation of lysozyme fibrils with different morphology in order to understand the mechanism of protein self-assembly. We have found that the stability of lysozyme is decreased in all studied ILs even for kosmotropic acetate and hydrogensulfate anions.

The mature fibrils were morphologically analyzed showing that both, anion and cation component of ILs modulate the morphology of fibrils, whereas the destabilizing effect of anions on lysozyme thermal stability is much more prominent than that of cations.

The work was supported by Slovak grant agency VEGA N. 2/0176/14, 2/0145/17, and SAS-MOST JRP 2015/5.

P-945

An insight into structure and stability of DNA in ionic liquids via molecular dynamics simulationK. Jumbri¹, M. B. Abdul Rahman², E. Abdul Malek², H. Ahmad², N. M. Micaelo³¹Universiti Teknologi Petronas, Malaysia; ²Universiti Putra Malaysia, Malaysia; ³Minho University, Portugal

Molecular dynamics simulation and biophysical analysis were employed to reveal the characteristics and the influence of ionic liquids (ILs) on the structural properties of DNA. Both computational and experimental evidence indicate that DNA retains its native B-conformation in ILs. Simulation data show that the hydration shells around the DNA phosphate group were the main criteria for DNA stabilization in this ionic media. Stronger hydration shells reduce the binding ability of ILs' cations to the DNA phosphate group, thus destabilizing the DNA. The simulation results also indicated that the DNA structure maintains its duplex conformation when solvated by ILs at different temperatures up to 373.15 K. The result further suggests that the thermal stability of DNA at high temperatures is related to the solvent thermodynamics, especially entropy and enthalpy of water. All the molecular simulation results were consistent with the experimental findings. The understanding of the properties of IL–DNA could be used as a basis for future development of specific ILs for nucleic acid technology

Posters

– 30. Ionic liquids meet biomolecules –

P-946 (O-176)

Ionic liquids vs biomembranes: a neutron scattering, atomic force microscopy and computational studyP. Kumari¹, C. Rotella¹, B. Rodriguez¹, A. Benedetto^{1,2}¹School of Physics, University College Dublin, Dublin 4, Ireland; ²Laboratory for Neutron Scattering, Paul Scherrer Institut, Villigen, Switzerland

The interaction between ionic liquids (ILs) and biomolecules is an emerging area of research. Among biological structures, model biomembranes such as phospholipid bilayers have been the first to be investigated in relation with ILs. The recognised affinity of ILs for phospholipid bilayers, first revealed in a short series of pioneering experiments and later confirmed by computer simulations and by neutron scattering experiments, opens a vast new playground to investigate the interaction of IL with paradigmatic biological structures. We will show that this far-reaching goal can be achieved by using neutron scattering as a structure- and dynamics-sensitive technique, and by resorting to atomic force microscopy (AFM) and molecular dynamics simulations. We will present recent experimental results on the microscopic mechanisms that allow ILs to penetrate lipid bilayers. Whereas neutron scattering allows to determine the characteristic time of the absorption of the single cations in the lipid region, which is of order of nanoseconds, AFM shows that cations penetrate first into the bilayer defects by following a power law distribution ($\beta=1.2$). Moreover, AFM allows to probe the mechanical properties of the lipid bilayers doped with ILs, which result more rigid than the net ones.

P-948

Model phospholipid self-assembly in ionic liquids and deep eutectic solventsA. S. Poulos¹, J. Griffith¹, L. McQueen², T. Welton¹, N. Brooks¹, O. Ces¹¹Imperial College London, UK; ²GlaxoSmithKline, USA

Ionic liquids (ILs) and deep eutectic solvents (DES) have emerged as a new class of solvents with unconventional properties. In view of their possible applications in the pharmaceutical industry, an improved understanding of their interactions with biological membranes is urgently needed.

Here we seek to understand the effect of ILs and DES on model phospholipid membranes by establishing ternary phase diagrams of lipid / IL-DES / water. Self-assembly and bilayer formation are investigated by polarized light optical microscopy and synchrotron small-angle X-ray scattering (SAXS). Pressure-temperature phase diagrams of lipids upon addition of ILs and DES are determined by high pressure SAXS. The phase boundary changes upon addition of ILs / DES reveal information about the structure and intrinsic curvature of lipid monolayers and bilayers. Moreover, pressure jump SAXS is employed to understand the kinetics of phase transitions between different mesophases in the presence of ILs / DES. These results quantitatively establish the effect of ionic solvents on model biomembranes, from both an equilibrium and non-equilibrium perspective. Our findings will permit a mechanistic understanding and modelling of this fundamental self-assembly process.

P-947

Computational modelling of enzymatic and colloid systems in room temperature ionic liquidsM. A. Mohammad Latif¹, M. B. Abdul Rahman^{1,2}¹Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, Malaysia; ²Enzyme and Microbial Technology Research Center, Universiti Putra Malaysia, Malaysia

We present a computational approach to study the effect of room temperature ionic liquids (RTILs) as non-aqueous solvents for enzyme and colloidal systems. Molecular dynamics (MD) simulation method was used to mimic the behaviour of several enzymes in [BMIM]-based RTILs with different anions. The structural stability and flexibility of all hydrolases studied in RTILs showed a dependency towards the water content. The solubility of protein structures in RTILs were estimated by solvation free energy calculations of amino acid side chain analogues. Non-polar analogues produced lower solvation free energy in hydrophobic anions while the polar ones showed better solvation in hydrophilic anions. RTILs were also investigated as a media for amphiphile self-assembly. MD simulations were performed to model the self-assembly process of Polysorbate 80 (T80) in water, [BMIM][PF₆] and [BMIM][BF₄]. Aggregation of T80 in RTILs was found significantly slower when compared with the one in aqueous solution. However, spherical aggregate in each solvents studied showed a comparable stability. Our current simulation results pointed towards the possibility of modelling a surfactant-coated enzyme for enhanced solubility in RTILs.

P-949

Evidence of Ionic Liquid induced bias in the disulfide bond isoform equilibrium of Conopeptides from a Molecular Dynamics studyD. Roy¹, K. A. Sajeevan¹¹Birla Institute of Technology and Science-Pilani, Hyderabad Campus, India; ²Birla Institute of Technology and Science-Pilani, Hyderabad Campus, India

Ionic Liquids (ILs) having numerous specialized features and structural variability have proven useful for biomolecular reactions. Here, we exploit ILs to control the disulfide bond isoform equilibrium observed in cysteine rich conopeptides. Conopeptides, the toxins found in marine cone snail venoms, attack ion channels and neuroreceptors and have huge therapeutic importance. When synthesized, these peptides yield several disulfide bond isoforms, structural elucidation and separation of which are challenging. Recent experiments indicate that oxidative folding of such peptides in certain ILs produce high yield of correctly folded native isoform. Using molecular dynamics we aim to understand the reason behind such a biological recognition. Our simulations reveal the general aspects of ionic solvation in biomolecules. The results suggest that in contrast to water, highly ionic and viscous solvents frustrate secondary structural motifs in peptides at low temperature. REMD simulations done on the peptides AuIB & GI in water and two typical ILs, [Im₄₁][Cl] and [Im₂₁][OAc], help scan the conformation space encompassing the disulfide bond isoforms. The results reveal strong solvent control in biasing the disulfide scrambling of AuIB/GI towards the native isoform in [Im₂₁][OAc].

Posters

– 30. Ionic liquids meet biomolecules –

P-950**Structure of proteins and phospholipid monolayers in deep eutectic solvents**

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Deep eutectic solvents (DES) are pseudo-ionic liquids obtained through the complexation of a halide salt with a hydrogen bond donor at a certain mole ratio. Recent investigations have revealed the ability of DES to sustain self-assembly of surfactants and they are now being investigated in enzymatic catalysis.[1,2] We have therefore begun an investigation on the structuring of complex amphiphiles in DES, in particular proteins and phospholipids.[3]

Here we report the conformation of proteins in pure and hydrated DES, and the behaviour of phospholipids at the air-DES interface. Circular dichroism and small-angle neutron scattering were used to investigate the structure of bovine serum albumin and hen egg-white lysozyme in choline chloride:glycerol DES. X-Ray and neutron reflectivity have provided an insight on the formation of stable, well defined DPPC and DMPC monolayers on the same solvent. Our results provide a novel approach to investigate these amphiphiles in the absence of water for these particular systems. Aiming to understand the fundamentals of the behaviour of biomolecules in DES, we will present details on protein conformation, as well as solvation effects in this complex media.

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Posters

– 31. Imaging molecules of life –

P-951

Fluorescent protein with fast spontaneous switching on kinetics for super easy nanoscopy

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We developed a photoswitchable fluorescent protein that exhibits quick spontaneous switching behavior from a fluorescence off state to an on state, and a large photon budget. These properties enable easy, fast and biocompatible super-resolution imaging based on single molecule localization microscopy without any complicated illumination set up. With this mutant fluorescent protein in conjunction with a conventional wide field microscope, we demonstrated superresolution imaging of cellular architecture at a 33 nm spatial resolution, 1 s time resolution (1 ms \times 1,000 frames for single image reconstruction of), and time-lapse imaging.

P-953 (O-185)

Single-molecule dynein, kinesin and IFT particle dynamics at the *C. elegans* ciliary tip

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Cilia are polar, microtubule-based cellular sensing hubs that rely on intraflagellar transport (IFT) for their development, maintenance and function. IFT trains, consisting of the IFT-A and IFT-B protein complexes and cargo, are co-operatively driven from ciliary base to tip by kinesin-2 motors. At the tip, trains turn around and are transported back to the base by IFT dynein. The mechanism of this turnaround at the tip has remained elusive. Here, we employ single-molecule fluorescence microscopy of IFT components in the tips of phasmid cilia of living *C. elegans*. Analysis of the trajectories reveals distinct turnaround behavior of different IFT components: while the motor proteins and IFT-A particle subcomplexes mostly turn around immediately, IFT-B particle subcomplexes show substantial pauses, lasting around 3 seconds. Our data provides the first *in vivo* single-molecule quantification of IFT tip turnarounds, providing new, comprehensive insights into this vital part of bidirectional transport in sensory cilia.

P-952 (O-184)

Peptide directed synthesis of continuous DNA nanowires for analysis of large DNA molecules with SEMK.-I. Kim¹, S. Lee², X. Jin², K. Su Ji¹, K. Jo², J. H. Lee¹¹Sungkyunkwan University, South Korea; ²Sogang University, South Korea

Being an important genetic molecule with programmable functionality, DNA is actively studied in diverse areas in cutting-edge science and technology. Although numerous studies have reported the visualization of DNA molecules, most studies were carried out with transmission electron microscopes, atomic force microscopes, or fluorescence microscopes, which are actually not desirable for whole analysis of micro/millimeter sized DNA molecules with nanometer sized features.

In this work, we developed a novel approach to synthesize smooth and continuous DNA nanowires applicable for analysis of large DNA molecules by SEM. By introducing thiol-tagged DNA binding peptides, we could densely introduce thiol groups into native DNA molecules. This allowed us to immobilize DNA molecules on conductive gold substrates and densely anchor gold nanoparticles onto DNA backbone. Subsequently, we could use these gold nanoparticles as seeds to guide smooth and continuous DNA templated metallization. Using our method, we not only could image smooth and uniform structures of long DNA (λ -DNA, 48.5 kb), its dimer (97 kb), and trimer (145.5 kb), but also observe entangled 3 dimensional images of DNA using SEM, which are very difficult to be achieved with other analytical techniques.

P-954 (O-186)

Near infrared fluorescent nanosensors for chemical imaging of chemical communication between cells

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Nanomaterials are versatile building blocks for fluorescent biosensors. We use carbon nanomaterials such as semiconducting single-walled carbon nanotubes (SWCNTs) as building blocks for such sensors. SWCNTs fluoresce in the near infrared (nIR) and their optoelectronic properties are very sensitive to changes in the chemical environment.

In general, a sensor requires a recognition unit but well-known motifs such as antibodies are often too large to achieve good sensitivity. Therefore we followed a new approach and created recognition motifs by letting smaller macromolecules such as DNA and DNA-peptide constructs directly adsorb and fold on the SWCNT surface. Using this concept we created different organic phases around SWCNTs and show that the best candidates are able to recognize certain biomolecules such as neurotransmitters or bacterial motifs.

Arrays of these sensors can be used for chemical imaging of small molecules. In order to predict the spatial and temporal resolution of this approach we additionally developed a Monte-Carlo based stochastic kinetic simulation and found that the rate constants play a decisive role. Finally, we show how such sensors can be used for nIR chemical imaging of neurotransmitter release by neuronal cells and pathogen detection.

Posters

– 31. Imaging molecules of life –

P-955

Directly watching biomolecules in action by high-speed atomic force microscopy

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Proteins are dynamic in nature and work at the single molecule level. Therefore, directly watching protein molecules in dynamic action at high spatiotemporal resolution must be the most straightforward approach to understanding how they function. To make this observation possible, high-speed AFM (HS-AFM) has been developed. Its current performance allows us to film biological molecules at 10–16 frames/s, without disturbing their function. In fact, dynamic structures and processes of various proteins have been successfully visualized, including bacteriorhodopsin responding to light, myosin V walking on actin filaments and even intrinsically disordered proteins undergoing order/disorder transitions. The molecular movies provided insights that could not have been reached in other ways. Moreover, the cantilever tip can be used to manipulate molecules during successive imaging. This capability allows us to observe changes in molecules resulting from dissection or perturbation. This mode of imaging was successfully applied to myosin V, peroxiredoxin and doublet microtubules, leading to new discoveries. Since HS-AFM can be combined with other techniques, such as super-resolution optical microscopy and tweezers, the usefulness of HS-AFM will be further expanded in the near future.

P-957

Interaction of an organo-osmium(II) anticancer complex with DNA probed at the single-molecule levelR. Berrocal-Martin¹, P. J. Sadler², S. W. Magennis¹¹WestCHEM, School of Chemistry, University of Glasgow, Joseph Black Building, Glasgow, G12 8QQ, UK;²Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

Metal-based drugs are key anticancer therapies and in many cases it is believed that the mechanism of cytotoxicity involves binding to DNA [1]. In order to develop new anticancer metallodrugs, it is important to understand how these drugs bind to DNA and what effect binding has on the DNA structure. Single-molecule techniques have revolutionised the study of biomolecular structure and function, including for DNA [2]. We will present the results of a single-molecule fluorescence investigation of the interactions of an osmium(II) arene complex, which is a promising class of anticancer drugs [3], and its interactions with DNA.

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P-956

Tracking and localization microscopy of single mitochondrial proteins in living cellsT. Appelhans¹, V. Wilkens¹, B. Rieger², G. Perkins³, K. Busch^{1,2}¹Mitochondrial Dynamics Group, School of Biology, University of Osnabrück, D-49076 Osnabrück, Germany; ²Institute of Molecular Cell Biology, School of Biology, University of Münster, D-48149 Münster, Germany; ³National Center for Microscopy and Imaging Research, University of California, San Diego, CA, USA

Mitochondrial function is closely connected to correct protein localization, interaction and organization. We recently demonstrated that protein organizations in the inner mitochondrial membrane show restricted mobility⁽¹⁾. Here, we use TIRF microscopy to image single mitochondrial proteins in an intact cell organelle to reveal the spatiotemporal organization of mitochondrial proteins, especially the OXPHOS proteins, on single molecule level in live cell. We established Tracking And Localization Microscopy (TALM)^(2,3) of post-translational, fluorescently labeled mitochondrial proteins reaching a calculated localization precision below 15 nm. Localization and tracking of mitochondrial proteins in living cells reveals the protein organization and diffusion behavior in submitochondrial microcompartments. Thereby we monitor OXPHOS proteins in cristae membranes and we provide evidence that the observed confinement of each protein is based on both, supercomplex formation and ultrastructural constraints. Additionally our results suggest a structure-function relationship in starving mitochondria.

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P-958

Fluorescent retinoids for cell biology and beyondD. R. Chisholm¹, C. Holden¹, R. Manning¹, K. Zmarzly¹, C. A. Ambler¹, R. Valentine², E. Pohl¹, A. Whiting¹¹Durham University, UK; ²High Force Research Ltd., UK

Retinoids are a class of molecules derived from Vitamin A that play key roles in a plethora of cellular processes, including differentiation, by mediating gene transcription in the nucleus. They have become key reagents in the preparation of cell cultures for use in the study of cell biology, and as powerful therapeutic agents. However, despite this, much is still to be learnt about the complex signalling pathway that governs their action. Endogenous retinoids like all-*trans*-retinoic acid (ATRA) are very vulnerable to photodegradation and isomerism due to their polyene structure, making their use as a research tool inherently uncertain. We have developed synthetic analogues of ATRA that are not only stable towards light, but are intrinsically fluorescent. These fluorescent retinoids can be uniquely imaged in a cellular environment using fluorescence microscopy – a property that has been exploited to reveal fascinating insights into their mode of action. Furthermore, their unique properties have enabled the development of a range of *in vitro* research tools, including a novel fluorometric binding assay. Our fluorescent retinoids can now be used to shed light on a signalling pathway that holds immense therapeutic potential, and whose influence is felt throughout the human body.

Posters

– 31. Imaging molecules of life –

P-959

Revealing protein oligomeric states in solution at the single molecule level with iSCATA. Fineberg, G. Young, D. Cole, N. Hundt, P. Kukura
University of Oxford, UK

Protein oligomerisation plays an essential role in many biological processes from formation of filaments to protein-ligand interactions. By elucidating the oligomeric populations of a protein, it is possible to gain mechanistic insights into its function in native and disease-related processes. Here, we show that interferometric scattering microscopy (iSCAT) can be used to directly determine the oligomeric state of proteins in solution without the use of any labels, one molecule at a time. Since the scattering intensity of a protein is proportional to its mass, we used proteins of known molecular weight to calibrate our mass measurements. We demonstrate the capabilities of single molecule solution mass spectrometry based on iSCAT to probe the nucleation of tubulin heterodimers leading to microtubule formation and growth.

P-961

Label-free visualisation of actin polymerisation using interferometric scattering microscopyN. Hundt, A. Tyler, G. Young, D. Cole, A. Fineberg, J. Andrecka, P. Kukura
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Actin filaments are a major component of the cytoskeleton and ubiquitously found in all cell types. Actin is involved in many basic processes such as cell migration, cell adhesion, cell division and muscle contraction. The most important property of actin is that it forms filaments (F-actin) out of globular subunits (G-actin). In the 1960s, Oosawa and co-workers developed a model for the G- to F-actin transition. The rate-limiting step of this transition is the formation of a stable actin nucleus, which was later determined to consist of 2-4 actin monomers. The nuclei are elongated by the addition of monomers to the filament ends. This model is widely accepted, although the molecular details could never be confirmed by direct observation.

Here, we used iSCAT to visualise the growth of unlabelled actin filaments. By applying a Gaussian worm model to fit the filament PSF we could follow the position of the filament ends. The advanced spatiotemporal precision of iSCAT allowed us to observe end displacements of ~2.7 nm, which correspond to the addition of single actin subunits. With these results we demonstrate the potential of iSCAT for label-free single-molecule imaging and for investigating the mechanisms of actin dynamics and their regulation.

P-960

3D millisecond tracking of single-molecule fluorescent protein translocation in eukaryotic cellsE. G. Hedlund¹, A. J. M. Wollman¹, S. Shashkova¹, S. Hohmann², M. C. Leake¹¹University of York, UK; ²Chalmers University of Technology, Sweden

Metabolic processes are the basis of all life. An organism must be able to utilise chemical energy to stay alive and eventually reproduce. These are the central features of life, regardless of organism length scale. To achieve this, an organism must be able to adapt to varying surrounding environmental conditions. Cells respond to external stimuli by releasing kinase cascades along often intricate signalling pathways which regulate cellular function. These chemical signals eventually bring about some cell level response.

In our research on transcription factor dynamics in Brewer's yeast, *Saccharomyces cerevisiae*, we have found that a key transcription factor, Mig1, forms functional clusters that translocate between nucleus and cytosol as a response to environmental glucose fluctuations (Wollman et al. 2017). The bulk behaviour of Mig1 glucose sensing in yeast has recently been well characterised (Bendrioua et al. 2014). However, the dynamics and interactions of individual molecules and clusters in the pathway have not.

By using astigmatic imaging at high speed, we can track fluorescently tagged proteins translocating in living cells over several tens of milliseconds. Using mutant yeast strains with fluorescent protein tags attached to the transcription factors Mig1. Furthermore, a microfluidic flow channel provides a consistent environment during an experiment.

P-962

Time-resolved imaging of oxidative stress and cell/tissue oxygenationV. Huntosova¹, S. Sokolova², M. Misuth², D. Horvath¹, G. Wagnieres³, P. Miskovsky^{1,4}¹Center for Interdisciplinary Biosciences, FS UPJS in Kosice, Jesenna 5, 041 54 Kosice, Slovakia; ²Dep. Biophysics, FS UPJS in Kosice, Jesenna 5, 04154 Kosice, Slovakia; ³Lab. of Organometallic and Medicinal Chemistry, ISIC, EPFL, Lausanne, Switzerland; ⁴SAFTRA photonics Ltd, Jesenna 5, 04154 Kosice, Slovakia

Cell metabolism is often altered in the process of cancerogenesis. O₂ imbalance can be measured by several techniques. Fluorescence lifetime spectroscopy is one interesting noninvasive approach to visualized malfunctions in cell metabolism. Unfortunately, many O₂ sensors such as porphyrins are used for these visualizations and have different levels of phototoxicity. We have compared different O₂ sensors phototoxicity, O₂ sensitivity, and ability to be used as a photosensitizer in photodynamic therapy (PDT). We have focused our works on mitochondria diseased cells with imbalanced respiration and metabolism. Hypoxic tissues exhibited dramatically decreased responses to PDT due to O₂ deprivation. We have also assessed the level of reactive O₂ species (ROS) by time-resolved fluorescence microscopy (FLIM) in cancer cells treated with regulators of apoptotic pathways affecting the mitochondrial network. The results were compared and analyzed by badging and boosting the advanced statistical methods that reduce non-Gaussian effects. Acknowledgement: the Slovak grants APVV 15-0485 and VEGA 1/0425/15 and by the Swiss NSF (project N° CR32I3_159746).

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P-963

AR3 and AR4 photoreceptor structures by serial X-ray crystallography at synchrotron and XFEL sources

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Retinal-containing photoreceptor proteins are found in all phyla of life. They undergo an ordered sequence of conformational changes in response to the absorption of light by the covalently bound chromophore.

Recently advances in XFEL and synchrotron beamline technology have enabled diffraction patterns to be obtained from streams of microcrystals in lipid cubic phase passed in front of an X-ray beam. By combining diffraction patterns from multiple crystals we have been able to obtain room temperature structures of the archaeobacterial proton pumps AR3 (a key protein for optogenetics) and AR4. Furthermore the rapid pulsing, high-intensity X-ray beam available at XFEL sources enables time-resolved pump-probe experiments to be performed in which the archaerhodopsin photocycle is stimulated by a laser pulse and the protein is allowed to change conformation before intercepting the X-ray beam. Diffraction data may therefore be obtained for later photocycle intermediates from wild-type, unmutated proteins.

P-965

Influenza virus vRNPs: quantitative investigations via fluorescence cross-correlation spectroscopy

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The influenza virus genome is composed of eight single stranded negative-sense RNAs forming viral ribonucleoprotein complexes (vRNP), within which the viral RNA is bound to the oligomeric nucleoprotein (NP) and the trimeric viral polymerase (PA, PB1, PB2), constituting an antiparallel double-helical structure. Following transcription and replication of vRNPs in the nucleus of infected cells, newly assembled vRNPs are exported in a CRM1 dependent manner with the assistance of the viral export complex consisting of the matrix protein 1 (M1) and the nuclear export protein (NEP). To date, neither the stoichiometry of export relevant viral proteins on single vRNPs, nor the selective genome assembly is entirely understood. Therefore, to advance understanding of the mechanism that influenza viruses use for nuclear export and genome assembly, we established an in vitro vRNP reconstitution assay and combined it with quantitative fluorescence cross-correlation spectroscopy (FCCS). Here we aim to quantify the viral export complexes on single vRNPs. Moreover, this setup allows us to investigate the sequence of vRNP assembly events in vitro. Eventually, we will extend and validate our preliminary findings in infection relevant cell lines directly.

P-964

High-resolution Atomic Force Microscopy (AFM) imaging of native biological membrane systems

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Many biological systems involve multiple membrane proteins and their arrangement strongly influences their function. *Rhodospirillum rubrum* chromatophore is an example photosynthetic organelle involved in the capture and conversion of solar energy, and its vesicular nature makes it challenging for AFM imaging. To image chromatophores tip-sample interaction needs to be minimized. Here, the imaging buffer was optimized to reduce long-range tip-sample interactions. Use of tapping mode AFM with high-resonance-frequency small and soft cantilevers, in combination with a high-speed AFM, reduced the forces due to feedback error. Despite the highly curved nature of the chromatophore membrane and lack of direct support, the above approach gave a resolution sufficient to identify the photosystem complexes and quantify their arrangement in the native state (Kumar *et al.*, *ACS Nano*, 2017, 11 (1)). Successive imaging of a chromatophore showed the proteins remain surprisingly static, with minimal rotation or translation. Novel, high order assemblies of RC-LH1-PufX complexes are observed, and intact ATPases are successfully imaged. The methods developed here are likely to be applicable to a broad range of protein rich native membrane systems, e.g., bacterial cell wall synthesis machinery.

P-966

Ultra-sensitive imaging and high-speed tracking with total internal reflection scattering microscopy

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Total internal reflection fluorescence (TIRF) microscopy has been widely used in single molecule tracking and imaging for more than 20 years due to its remarkable background suppression and high axial resolution below the diffraction limit. Some intrinsic photophysical properties of fluorescent labels, however, such as photobleaching and photoblinking, as well as the presence of biological auto-fluorescence, impede the application of TIRF to particle tracking problems, especially those requiring high spatiotemporal precision. Here, we present a micro-mirror total internal reflection dark field scattering microscopy (tirSCAT) with unprecedented levels of background suppression and spatiotemporal resolution. Our approach uses micromirror-based coupling of incident and reflected illumination light with optimised mirror diameters and objective mirror separation. Using this approach, we demonstrate tracking of gold nanoparticles down to 10 nm diameter with simultaneous nm precision and temporal resolution on the order of 10 microseconds at incident power densities comparable to single molecule fluorescence imaging, opening up new avenues to studying structural dynamics and motion of biological macromolecules.

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P-967

Introducing the new Cypher VRS: a video rate atomic force microscope

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In this presentation we will introduce our new Atomic Force Microscope (AFM), the Cypher VRS. This new video rate AFM sets a new benchmark for speed, enabling high resolution imaging of dynamic events at up to 625 lines/second (10 frames per second). This is about 300x faster than typical AFMs and 10x faster than current “fast scanning” AFMs. The Cypher VRS is the only AFM to achieve these speeds while still offering the versatility and ease of use of a full-featured research AFM. It's ideal for researching dynamic events such as biochemical reactions, membrane studies, self-assembly, and boundless other applications in materials and life science. We will describe the architecture of this new AFM, and demonstrate the advantages of its unique design (speed, resolution, ease of use, versatility of samples and applications...). In this presentation we show such examples as cleavage of DNA molecules by the enzyme DNase1, the real time structure and arrangement of Bacteriorhodopsin molecules and the assembly of type I collagen molecules into fibrils.

P-969

Molecular mechanisms of diseases revealed by single molecule imagingA. Palmer¹, B. Gole², R. Wiehe², M. Reisser¹, A. Cato³, L. Wiesmüller², J. C. M. Gebhardt¹¹Institute of Biophysics, Ulm University, Germany; ²Division of Gynecological Oncology, Ulm University, Germany;³Institute of Toxicology and Genetics, KIT, Germany

Many severe diseases can be traced back to a malfunctioning molecular specie, evoked for example by a mutation. Although often challenging, additional identification of the disease-causing molecular mechanism would greatly facilitate developing therapeutics. We here use single molecule tracking (SMT) to uncover the malfunctions of molecules causing two different diseases.

Spinobulbar muscular atrophy originates from a pathogenic increase in length of the polyQ sequence of androgen receptor (AR). Using SMT, we found that long-lasting specific interactions between the pathogenic mutant (Q65) and DNA were strongly reduced compared to wild type (Q22). This suggests a mechanism of hampered transcriptional activation of Q65 contributing to the disease.

In secondary leukemia, the mitochondrial protein endonuclease G (EndoG) mislocalizes to the nucleus upon chemotherapy, where it facilitates rearrangements of the Mixed Lineage Leukemia (MLL) gene. Recently, Gole and Wiesmüller identified a potential inhibitor of EndoG-induced DNA restriction. Using SMT, we found that the inhibitor functions by reducing EndoG-DNA associations. Further optimization of the molecule inhibiting EndoG might result in improved cancer therapies.

P-968

Focusing Single-Molecule Localization Microscopy on single-cell biologyR. Nafiseh¹, D. Nino², A. Zilman^{1,2}, J. N. Milstein^{1,2}¹Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada; ²Department of Physics, University of Toronto, Canada

Cell biology is becoming increasingly quantitative with advances in light microscopy strongly driving this trend. Beyond imaging structure, significant effort has gone into developing microscopy based approaches to determining the abundance of proteins and nucleic acids in cells. Molecular counting experiments can yield additional insight into cellular structure and define the stoichiometry of interacting protein complexes. Moreover, since microscopy provides information at the single-cell level, it may be used to study stochastic variation within a population due to varying levels of mRNA and protein copy number, which is inaccessible to bulk techniques. Single-molecule localization microscopy (SMLM) has the potential to serve as an accurate, single-cell technique for counting the abundance of intracellular molecules. However, the stochastic blinking of single fluorophores can introduce large uncertainties into the final count. Here we provide a theoretical foundation for applying SMLM to the problem of molecular counting based on the distribution of blinking events from a single fluorophore.

P-970

Visualisation of DNA conformational changes in situ at nanometre resolutionA. L. B. Pyne¹, B. W. Hoogenboom¹, R. Vilar², A. Maxwell³¹London Centre for Nanotechnology, University College London, WC1H 0AH, UK; ²Department of Chemistry, Imperial College London, SW7 2AZ, UK; ³Department of Biological Chemistry, John Innes Centre, Norwich, NR4 7UH, UK

Non-canonical DNA structures are implicated in key biological processes such as gene regulation and chromosome maintenance, and often introduce genetic instabilities. These sequences also offer targets for novel therapeutics. By embedding such sequences in small circles of DNA, we can study the effect of topological constraint on the formation of alternative DNA structures. Here we use high resolution AFM to visualise both DNA structure and superstructure, thereby determining the supercoiling and helical pitch of each individual molecule. For minicircles containing a triplex forming sequence, we observe triplex formation as nanometre sized protrusions from the DNA minicircle, across a range of superhelical densities. In quadruplex-containing DNA minicircles, we distinguish between minicircles with unfolded ssDNA sections and those folded into quadruplex DNA¹. We also observe quadruplex formation in real time, with folding and unfolding occurring within one minute. Complementary FRET experiments confirm that DNA minicircles provide a more physiological alternative for the evaluation of novel therapeutics such as quadruplex ligands.

1) Klejevska, B. et al. Chem. Commun. 2016, 52 (84), 12454–12457.

Posters

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P-971

High-speed AFM imaging of the MinDE protein oscillatorB. Ramm¹, A. Miyagi^{2,3}, S. Scheuring^{2,3}, P. Schwille¹¹Max Planck Institute of Biochemistry, Germany; ²Aix-Marseille Université, France; ³Weill Cornell Medicine, USA

The *E. coli* MinCDE system has become a paradigm for spatiotemporal pattern formation. Based on the ATPase MinD and its activator MinE, this minimal biological oscillator defines the midcell in *E. coli*. In vitro reconstitution of MinDE on supported lipid bilayers in planar or cell-shaped geometry leads to travelling surface waves or pole to pole oscillations, respectively. Despite intense study the molecular mechanism of MinDE oscillations is still not entirely understood.

Here, we employed high-speed atomic force microscopy (HS-AFM) to probe MinDE dynamics on nanometer sized, isolated membrane patches. HS-AFM imaging enabled us to observe MinDE association and dissociation cycles of individual point oscillators in the absence of mesoscale patterns. Analysis of the kinetics with varying MinD and MinE protein concentrations and dependent on membrane patch size allowed us to discern the different phases in the oscillation cycle. We show that both the association and dissociation are highly cooperative and cross between remarkably long lasting states in which the membrane is either fully protein covered or empty. Based on our results, we propose that association and dissociation represent the two directions of a single allosteric switch leading to MinD filament formation and depolymerization.

P-973

Single amyloid aggregates chemical and structural analysis by infrared nanospectroscopy

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A wide class of neurodegenerative disorders is due to the failure of a peptide or protein to keep its native functional conformational state and undergo a conformational change into a misfolded state, triggering the formation of fibrillar cross- β sheet amyloid aggregates. Despite its fundamental role in biological function and malfunction, the mechanism of protein self-assembly and its link with cellular toxicity and neurodegeneration remains challenging to elucidate in molecular detail. Mainly because unraveling amyloid species biophysical properties, which possess nanoscale dimensions and heterogeneous nature, represents a formidable experimental challenge.

Here, we demonstrate that Atomic Force Microscopy (AFM) techniques can measure the properties of heterogeneous populations and investigate the biophysical properties of amyloid aggregates at the single molecule nanometer scale. In particular, we establish Infrared Nanospectroscopy, simultaneously exploiting AFM and Infrared Spectroscopy, as a versatile tool to characterize at the nanoscale the conformational rearrangements of proteins and their polymorphic aggregation. This information is central to design molecules that could interfere with amyloid aggregation delaying the onset of misfolding diseases.

P-972

Diffusion of gold-tagged lipids as comparison benchmark for iSCAT and fluorescence-based experimentsF. Reina^{1,2}, P. Kukura², C. Eggeling¹¹Eggeling Nanoimmunology Lab, MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK; ²Kukura Lab, Department of Chemistry, University of Oxford, UK

The study of the organization and dynamics of molecules such as lipids in the cellular plasma membrane is an important topic in contemporary Biophysics. These studies have proven particularly challenging as they demand simultaneous high spatial and temporal resolution. Here we compare two suitable approaches, based on Fluorescence Correlation Spectroscopy and super-resolution optical STED microscopy (STED-FCS), and on Interferometric Scattering (iSCAT) microscopy. While STED-FCS requires labelling with small fluorophores, iSCAT usually requires the use of scattering tags, such as 5–40 nm functionalized gold nanoparticles. The use of such large labels may alter or introduce spurious effects in the diffusion behaviour of the target lipids. We used several lipid analogues on a fluid Supported Lipid Bilayer (SLB), and compared their diffusion modes when tagging them with a small fluorophore or with a fluorescently labelled gold nanoparticle. STED-FCS and iSCAT measurements proved that tagging with 40 nm gold nanoparticles reduced the diffusion speed of the lipids but did not introduce anomalous diffusion dynamics. These results provide a promising basic protocol for future STED-FCS and iSCAT experiments on more complex model systems and live-cell membranes.

P-974

Imaging complement by phase-plate cryo-electron tomography from initiation to pore formationT. H. Sharp¹, A. J. Koster^{1,2}, P. Gros³¹Leiden University Medical Center, Netherlands; ²NeCEN, Leiden University, Netherlands; ³Utrecht University, Netherlands

Phase plates in cryo-electron tomography improve contrast, increasing the ability to discern separate molecules and molecular complexes in dense biomolecular environments. Here, we apply this new technology to the activation of the human complement system, an important immune defense against bacteria. Binding of C1 to antibody complexes initiates a proteolytic cascade that deposits molecules onto adjacent surfaces and terminates with formation of membrane attack complex (MAC) pores in the targeted membranes. The data shows instances of C1 transiently interacting with its product C4b, as well as formation of MAC pores through sequential binding and membrane insertion of C5b6, C7, C8, and multiple copies of C9. Using subtomogram averaging, we determined the C5b-9 pore structure in lipid bilayers at 2.3 nm resolution, revealing a cone-shaped pore that is poorly closed, yielding a seam between C9 and C6. Large variations of composite pore complexes are apparent in subtomograms. Oligomerized initiator complexes C5b-7 and C5b-8 show stages of membrane binding, deformation, and perforation that yield ~3.5 nm-wide pores. These data indicate a dynamic process of pore formation.

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– 31. Imaging molecules of life –

P-975

Transverse fluorescence microscopy with magnetic and optical tweezers

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Magnetic and optical tweezers are a key tool when examining single molecules of DNA. However, it has not so far been possible to directly manipulate DNA through an applied force or torque while imaging its full contour length. In this work a novel magneto-optical tweezing tool built at the University of York has been combined with superresolution microscopy and an experimental geometry in which the DNA is held horizontally to the field of view. One end of the DNA is bound to an anchor bead on the coverslip through DIG-antiDIG interactions, while the other is attached to a smaller superparamagnetic bead with biotin-streptavidin bonds. The free bead may then be trapped with a near-IR laser, rotated with a magnetic field, or both. Back focal plane detection using the IR laser quantifies trap stiffness and rotational frequency, as well as applied force. The DNA is imaged with the intercalating dye YOYO-1, which may be localised to ~30-40nm, and can be compared to multiscale simulations to give detailed information about molecular structure. This methodology will allow real-time imaging and quantification of key DNA processes such as plectoneme formation or overstretching, and the apparatus may also be used to monitor molecular machines with a view toward bottom-up drug design.

P-977

Imaging endogenous activation-induced cytidine deaminase (AID) regulation in mammalian B cells

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In activated B cells, AID deaminates cytosine to uracil on immunoglobulin (Ig) genes to initiate somatic hypermutation and class switch recombination, which are essential for antibody diversification. Tight AID regulation is crucial because off-target deaminations can generate spurious mutations that may lead to cancer. To understand the mechanisms of AID targeting to Ig genes specifically, we have developed a mouse B cell line where endogenous AID was tagged with eGFP by CRISPR/Cas9. Using STORM, we track the dynamics of AID molecules in response to antigen activation, which reveals that, while AID is predominantly cytosolic, a few AID molecules dynamically translocate to the nucleus for function. In addition, using cryo-thin sectioning microscopy, we also find that AID co-localises primarily with initiating RNAP II, but not with elongating RNAP II in activated B cells. Our data support a model whereby AID recruitment occurs primarily at active gene promoters.

P-976

The problem of measurement in cell biology: A tale of two alleles

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Fluorescence reporters allow investigation of temporal changes in protein expression in live cells and are consequently an essential measurement tool in modern molecular biology. However, their utility is dependent on their accuracy, and the effects of reporter constructs on endogenous gene expression kinetics are not well understood. Here, using a combination of mathematical modelling and experiment, we show that widely used reporter strategies can systematically disturb the dynamics they are designed to monitor, sometimes giving profoundly misleading results. We illustrate these results by considering the dynamics of the pluripotency regulator Nanog in embryonic stem cells, and show how reporters can induce heterogeneous Nanog expression patterns in reporter cell lines that are not representative of the wild-type. These findings help explain the range of published observations of Nanog variability and highlight the problem of measurement in cell biology in relation to genetic reporters.

P-978

A combinatorial single-molecule study of ligand-gated ion channels and monoclonal antibodies

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Ion channels are critical modulators of cell function, implicated in many pathophysiologicals. Mechanistic insight into the interactions of these receptors with ligands will be critical in the discovery of novel drugs to target them. Atomic force microscopy (AFM) is a powerful tool for single-molecule studies in this area. Here we describe methods to study ligand-gated ion channels and monoclonal antibodies by AFM. Receptors are imaged directly adsorbed onto a mica substrate or reconstituted into supported lipid bilayers that act as model membranes. Receptors adsorbed on functionalised mica appear vertically compressed, whilst reconstituted receptors have dimensions consistent with crystal structures and can be stimulated by the addition of a ligand. Immobilised antibodies are imaged at sub-molecular resolution in liquid, resolving individual domains within antigen-binding fragments. It has thus far proven non-trivial to obtain real-time AFM images of receptor-antibody interactions in fluid. This work sheds light on issues encountered during AFM imaging of therapeutically-relevant complexes, which are often weakly bound and/or highly transient. These methods will be the framework of an AFM-based drug assay applicable to many ion channels.

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– 31. Imaging molecules of life –

P-979**Single-Molecule observation of CRISPR-Cas9 dynamic behaviour in *Escherichia coli***

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CRISPR-Cas9, which is the popular enzymatic complex that produces DNA double-strand breaks when associated with a guide-RNA, has recently become a widely-used synthetic biology tool, allowing easy and efficient gene editing. However, the dynamics of this nucleo-proteic complex are not fully understood *in vivo*. For instance, we still do not know Cas9's residence time on the DNA, when ongoing off- or on-target activity. Furthermore, Cas9's potential interactions with other DNA-associated proteins are similarly unknown. This information would be particularly relevant in the context of DNA repair, which appeared as an essential mechanism through which CRISPR-Cas9's wide range of activities can be expressed. Here, we propose to address these questions using single-molecule fluorescent microscopy in association with computational localization and tracking to follow Cas9 behaviour in *Escherichia coli* over time, space and along DNA.

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Posters

– 32. Receptors and signalling –

P-980 (O-190)

Impact of membrane lipid composition on Dopamine D2 receptor activation

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The dopamine D2 receptor (D2R) is a 7-transmembrane receptor that strongly interacts with membrane lipids, however, the implication of the membrane lipid environment on D2R properties has been overlooked. Yet, the brain is highly enriched in lipids and in polyunsaturated fatty acids (PUFAs) in particular, which are known to impact the properties of membranes as well as the activity of transmembrane proteins. Recent *in vivo* data obtained in the lab suggest that membrane PUFA content impacts the functionality and signaling properties of the D2R. Our project aim was to unravel the impact of membrane lipid composition on D2R conformation and pharmacological properties through biophysical studies in both cell membrane fragments and membrane models systems. To this aim, we have performed *in cellulo* and *in vitro* studies using fluorescence anisotropy and plasmon waveguide resonance (PWR) on cells enriched with specific PUFAs and in lipid reconstituted model systems of controlled lipid composition. Overall the data indicates that n-3 and n-6 PUFA enhance both agonist and antagonist affinity for D2R. The results could have a significant impact in the development of novel therapeutic strategies for psychiatric disorders in which the D2R plays a key role.

P-982 (O-192)

Proton-induced conformational switching in GPCRs is tailored to the membrane interface

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Ionic and H-bond interactions between the cytosolic ends of transmembrane helices TM3 and TM6 of class-A (rhodopsin-like) G protein-coupled receptors (GPCRs) stabilize the inactive receptor conformation. In the photoreceptor rhodopsin, proton uptake at Glu134 in the conserved E(D)RY motif breaks this "ionic lock" leading to receptor activation. Our MD calculations on membrane-embedded TM3 peptides show that protonation of the conserved glutamic acid alters the side chain rotamer preference and stabilizes the C-terminal helical structure. The altered peptide topology at the membrane interface rises of the side chain pKa (> 6) and lowers the polarity around the TM3 C-term as revealed also by fluorescence spectroscopy. The effects were not seen with an amide. Also time-resolved FTIR spectroscopy showed different kinetics for lipid ester carbonyl hydration around TM3, suggesting that the carboxyl is linked to a more extended H-bond network than an amide at the same position. The same was seen in DOPC-reconstituted opsin mutants (carrying a Glu134 or Gln134). Thus, the E(D)RY motif is a proton-regulated hydrated membrane microdomain. It acts as a proton switch through reorganization of the water H-bond network at the membrane interface.

P-981 (O-191)

Unraveling the mystery of the seemingly too short linker in bivalent ligands of opioid receptors

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Linking G-protein coupled receptor (GPCR) dimers by bivalent ligands (BLs) is an elegant method for studying and altering biological activities of GPCRs [Shonberg et al, *ChemMedChem* 2011]. BLs with the largest pharmacologic impact target an opioid heterodimer consisting of inactive receptor δ (DOR) and active receptor μ (MOR*). Thereby, tolerance and dependence caused by MOR agonists (e.g. morphium) is significantly reduced [Daniels et al, *PNAS USA* 2005]. A common opinion is that the individual ligands are buried in the orthosteric pockets of the receptors while the linker is located in the extracellular space. However, such linkers would need to be 4-5 nm long [Glass et al, *TrendsPharmacolSci* 2016] which contradicts the functionality of multiple known BLs with linkers shorter than 2.5 nm. Here by multiscale molecular dynamics simulations, we studied the required linker length connecting a μ agonist and a δ antagonist [Harvey et al, *ACS MedChemLett* 2012] in different DOR/MOR* dimers. In the most potent dimers the ligands were connected by a 2 nm-long linker. The stability of the ligand in the subsequent atomistic simulation confirms that BLs with short linkers (~2 nm) can bind two GPCRs simultaneously. Thereby, the linker passes between two helices directly to the next receptor.

P-983

Nano-clustering of ligands on synthetic APCs influences T-cell membrane and actin organization

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The interface between an Antigen Presenting Cell (APC) and a T-lymphocyte (T cell), plays a key role in sensitivity and precision of antigen recognition by T-cells. It has recently been shown that the antigens (in form of ligands for T-cell receptor-TCR) are present on the membrane of APCs in the form of submicronic clusters. Here we mimic this aspect of the APC-membrane by presenting molecules of anti-CD3 (targeting the TCR-complex), in the form of an array of submicrometric dots surrounded by a sea of polymers or by a fluid supported lipid bilayer (SLB), which is optionally functionalized with ICAM-1 (ligand for the T-cell integrin-LFA) [Nanolett2015]. TCR and ZAP-70 clusters, actin in form of puncta, and zone of membrane tight adhesions all colocalize with the ligand nano-dots; however global cell spreading area depends only on average ligands density [IntgBiol2016]. In presence of ICAM-1, TCR/ZAP70 organization remains the same, but actin goes from punctate to peripheral ring organization, and the membrane goes from being topographically patterned to flat. Interestingly dynamic imaging hints that TCR organizes the actin in early time and LFA-1 at late time, thus pointing to the crucial but different role of both in T cell spreading and activation.

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P-984

Real time monitoring of membrane GPCR reconstitution by plasmon waveguide resonanceP. Calmet^{1,3}, M. de Maria², E. Harté³, A. Jazayeri⁴, N. Tschammer⁵, I. D. Alves³¹Max Planck Institute for the Science of Light, Germany; ²Friedrich Alexander University (FAU) Erlangen-Nürnberg, Germany; ³Chemistry and Biology of Membranes and Nanoobjects, France; ⁴Heptares Therapeutics Ltd., Germany; ⁵NanoTemper Technologies GmbH, UK

G-protein coupled receptors (GPCRs) are important therapeutic targets since more than 40% of the drugs on the market exert their action through these proteins. To decipher the molecular mechanisms of activation and signaling, GPCRs often need to be isolated and reconstituted from a detergent-solubilized state into well-defined and controllable lipid model systems. Several methods exist to reconstitute membrane protein in lipid systems but usually the reconstitution success is tested at the end of the experiment and often by an additional and indirect method. Irrespective of the method used, the reconstitution process is often an intractable and time-consuming trial-and-error procedure. Herein, we present a method that allows directly monitoring the reconstitution of GPCRs as well as other membrane proteins in model planar lipid membranes using the chemokine receptor CCR5 as an example. Plasmon waveguide resonance (PWR) allows following GPCR lipid reconstitution process without any labeling and with high sensitivity. We go on to prove the usefulness of the techniques by studying the effect of different lipid contexts on antagonist binding to the chemokine CCR5 receptor.

P-986

Dynamic tuneable G protein-coupled receptor monomer-dimer populationsP. M. Dijkman^{1,3}, O. K. Castell^{1,4}, A. D. Goddard^{1,5}, J. C. Muñoz-García^{1,6}, C. de Graaf², M. I. Wallace¹, A. Watts¹¹University of Oxford, UK; ²Vrije Universiteit Amsterdam, Netherlands; ³Max Planck Institute of Biophysics, Germany; ⁴Cardiff University, UK; ⁵Aston University, UK; ⁶University of East Anglia, UK

G protein-coupled receptors (GPCRs) play a pivotal role in cellular signalling, highlighted by the fact that they form the target for ~40% of pharmaceuticals. While evidence has been accumulating for the existence and functional significance of GPCR oligomers, the matter remains controversial, in part due to lack of consensus on *e.g.* the receptor interfaces involved in oligomerisation, and their possible dynamic nature [1]. Neurotensin receptor 1 (NTS1) has previously been shown to dimerise in lipid bilayers [2], is one of few GPCRs that can be produced in *E. coli*, and holds therapeutical potential for a variety of conditions including schizophrenia and cancer. Using a combination of single-molecule [3], and ensemble FRET, DEER spectroscopy, and Monte Carlo and molecular dynamics simulations, we demonstrate the presence of a concentration-dependent dynamic equilibrium between NTS1 monomers and dimers, with multiple co-existing dimer interfaces. These findings could rationalise previous seemingly contradicting results, and may provide a means of regulation of receptor signalling *in vivo*. [1] Ferré et al. (2014) *Pharmacol Rev* **66**; [2] P.J. Harding et al. (2009) *Biophys J* **96**; [3] Leptihn et al. (2013) *Nat Protoc* **8**

P-985

X-ray single molecule dynamics of interleukins bonded receptors on live NK cells membranesJ.-W. Chang^{1,2}, Y. S. Baek³, K. Mio², M. Mio², H. Sekiguchi⁴, Y. C. Sasaki^{1,2}¹The University of Tokyo, Japan; ²OPERANO-OIL AIST/UT, Japan; ³CHA University, South Korea; ⁴SPRING8/JASRI, Japan

We succeeded in measuring different dynamics of Interleukin-2 (IL-2) and Interleukin-15 (IL-15) bounded to their receptors on Natural Killer (NK) cells membranes using x-ray single molecule technique (Diffracted X-ray Tracking: DXT) at the sub-second level. The activated NK cell capable of anticancer have been hitherto paid attention. There are unidentified different dynamics of IL-2 and IL-15 with their receptors penetrating NK cell membrane supposed to determine the different second signals of immune systems. Many researches about biochemical functions have been executed about these almost same IL-2 bounded IL-2 receptor (IL-2/IL-2R) and IL-15 bounded IL-2 receptor (IL-15/IL-2R). Until now, the different dynamics between these supramolecules supposed to be made different function have never been evaluated. In this study, we tracked diffractions of gold nanocrystals tethered to IL-2/IL-2R and IL-15/IL-2R on live NK cells in order to measure dynamics of initial states inducing NK cell activation using both laboratory X-ray source (Rigaku, FR-D) and synchrotron orbital radiation source (BL-40XU, SPRING-8) at the sub-second level. DXT can observe dynamical rotational motions in the individual single functional proteins using the time-resolved Laue diffraction technique.

P-987

Direct visualization of APLP1 cell-cell adhesion platforms via fluorescence fluctuation spectroscopyV. Dunsing¹, M. Mayer², G. Multhaup², S. Chiantia¹¹Universität Potsdam, Germany; ²McGill University, Canada

Amyloid precursor-like proteins (APLPs) play a key role in nervous system development and are involved in the pathology of Alzheimer's disease. Here, we investigate the cellular function of APLP1. We performed 2 color scanning fluorescence correlation spectroscopy (2csFCS) and cross-correlation Number&Brightness (ccN&B) on living cells. At cell-cell contacts, we observe a significant spectral cross-correlation, thus evidence of direct APLP1 trans interactions. Recently, we have shown that μ M zinc concentrations trigger the formation of APLP1 multimers at the plasma membrane. Here, we report the enrichment of large APLP1 clusters at cell-cell contacts upon zinc addition with enhanced trans interactions. These clusters are characterized by very slow dynamics, i.e. diffusivities of $10^{-3} \text{ } \mu\text{m}^2/\text{s}$. Finally, we study APLP1 interactions in Giant Plasma Membrane Vesicles and find that APLP1 clusterization requires the presence of the cytoskeleton. Taken together, our results strongly indicate that i) APLP1 functions as a neuronal adhesion receptor, ii) zinc modulates APLP1 adhesion and mediates the formation of large, static adhesion platforms at cell-cell contacts, iii) 2csFCS and ccN&B are promising tools to study cell-cell, i.e. protein-protein trans interactions in living cells.

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– 32. Receptors and signalling –

P-988

Triggering of the high-affinity IgE receptor in an aggregation-independent mannerJ. H. Felce^{1,2}, E. Sezgin¹, M. Wane¹, M. L. Dustin², C. Eggeling¹, S. J. Davis¹¹Weatherall Institute of Molecular Medicine, University of Oxford, UK; ²Kennedy Institute of Rheumatology, University of Oxford, UK

The high-affinity IgE receptor, FcεR1, is responsible for the sensitization of mast cells and basophils to IgE-targeted antigens. The current model of FcεR1 triggering rests on the principle of aggregation-driven phosphorylation, yet several recent observations have indicated that this is incomplete. Here we re-examine the minimal requirements for FcεR1 triggering and observe that it is induced without receptor aggregation by surface-associated antigen in a size-dependent manner. Using confocal microscopy and fluorescence correlation spectroscopy we determine that this is driven by exclusion of the large inhibitory phosphatase CD45 from the receptor-antigen contact, abrogation of which inhibits triggering. Such kinetic segregation (KS) of CD45 and FcεR1 also occurs in triggering-deficient cells and basophil-derived giant plasma membrane vesicles, and hence is a passive, steric process. Partial KS also occurs in cells forming surface contacts in the absence of FcεR1 ligand, leading to ligand-independent triggering that is responsible for the conventional morphology of basophilic cell lines in culture. The potential for both aggregation and KS mechanisms broadens the range of antigen capable of inducing triggering, and so affords the system substantially increased versatility.

P-990

Some positively charged aminoacids are essential for the binding of C1B domain of PKCε to membranes

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C1 domains are members of the Cys-rich domains superfamily, formed by 50–51 amino acids residues present in many types of proteins, as it is the case of the classical and novel Protein Kinases C (PKCs). Both types of PKCs, classical and novel isoenzymes, possess two C1 subdomains, C1A and C1B, although it is not totally clear why two modules are needed. C1 domains are known to interact with diacylglycerol and phorbol esters. Since we have shown previously that the interaction of the C1B domain of PKCε with the membrane depend not only on interaction with diacylglycerols but also on the interaction with positively charged residues we have tried to identify which residues are important for this interaction. K251, R268, R282 and R283 were replaced by Ala and we characterized the effect of single, double and triple mutations. Results show that binding is decreased by increasing of the number of residues mutated in the domain, and it be even abolished in the presence of diacylglycerol. In conclusion, the electrostatic interactions derived of these positively amino acids residues are important for membrane docking which is further stabilized by interaction with the diacylglycerol.

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P-989

Homo- and heterodimerization of G protein coupled chemokine receptors

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Chemokine receptors are involved in cancer metastasis as well as HIV-infection and were observed to form homo- and heterodimers. The receptors CXCR4, CCR2 and CCR5 were shown to homo- and heterodimerize and the receptor association was reported to regulate the proteins' function. In addition, the presence of membrane cholesterol was observed to be essential for receptor activity.

We employed thousands of molecular dynamics simulations on the microsecond timescale to study the dimerization of these receptors in absence and presence of cholesterol.

We discovered that the homo- and heterodimerization of CXCR4 is strongly affected by cholesterol. Cholesterol binding to the transmembrane helix 1 (TM1) partially blocks this helix from dimer interactions, whereas cholesterol intercalation between the receptors induces the formation of homo- and heterodimers with TM4 of CXCR4 at the dimer interface [Pluhackova and Gahbauer et al., *PLOS Comput. Biol.*, 2016].

Interestingly, blocking the TM4 of CXCR4 with specific peptides in other experiments was shown to modulate homo- and heterodimers and to reduce CXCR4 function.

In summary, our results connect the former experimental observations where cholesterol and TM4 of CXCR4 were reported to be crucial for the function of chemokine receptor dimers.

P-991

Interactions between the oncoprotein E5 and PDGF receptor revealed by solid-state ¹⁹F-NMR distance measurements in membranes

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The oncogenic E5 protein from papillomavirus is able to activate the platelet-derived growth factor receptor (PDGFR) through interaction within the bilayer, which leads to uncontrolled cell proliferation and cancer. This activation is initiated solely by interaction of E5, which forms a homo-dimer of two transmembrane helices, with the transmembrane helix pair of the PDGFR dimer.

Our aim was to elucidate the structure of the E5-E5 dimer interface, and changes upon PDGFR binding, to get clues for the mechanism of the activation of PDGFR by E5 in the membrane. For this aim we have previously developed a solid-state ¹⁹F-NMR strategy to determine long-range intermolecular distances. Using the CPMG experiment on oriented bilayer samples, we have been able to address distances up to 11 Å in fluid membranes. When applied to the E5 dimer, we revealed inter-molecular contacts in fluid membranes between selective ¹⁹F-labels which were placed in one out of five positions along the E5 helix. Using CODEX on immobilized E5, the dimer interface could be mapped in detail and changes upon PDGFR binding could be monitored. Based on our results, we derived a viable model of the E5 dimer in lipid membranes and hints for its interaction with the PDGFR receptor.

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– 32. Receptors and signalling –

P-992

G protein-coupled receptor lipid interactions: Insights from molecular dynamics simulationsG. Hedger¹, H.-Y. Yen², M. Horrell¹, I. Liko², C. V. Robinson², C. Siebold³, M. S. P. Sansom¹¹Department of Biochemistry, University of Oxford, Oxford, UK; ²Department of Chemistry, University of Oxford, Oxford, UK; ³Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, UK

We have undertaken multiscale molecular dynamics simulations to investigate two distinct modes of lipid interaction with G-protein coupled receptors (GPCRs). Native mass spectrometry measurements suggest the presence of specific lipid binding sites on Neurotensin Receptor 1 (NTS1), in particular for phosphatidyl inositol phosphate (PIP) species. Coarse-grained molecular dynamics simulations of NTS1 embedded in a lipid bilayer indicate strong and specific interactions of PIP molecules with defined regions on the intracellular portion of NTS1. Extending the simulations to a range of available GPCR structures suggests a degree of conservation. Molecular simulations have also been used to explore interactions of cholesterol with the extracellular domain of Smoothed (SMO), a Class F GPCR. The recent near full-length x-ray structure of SMO revealed the presence of a cholesterol molecule within a hydrophobic sterol binding groove on the extracellular cysteine rich domain (CRD). Atomistic simulations of SMO in a lipid bilayer suggest the cholesterol molecule exerts a marked effect on the structural stability of the CRD, as well as the potential for a degree of flexibility of extracellular domains of SMO relative to 7TM domain.

P-994

ACE, NOS3 and GST (M1 & T1) genes polymorphisms and the risk of myocardial infarction in Bangladesh

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The aim of the present study was to find out the associations of ACE insertion/deletion (I/D), NOS3 (G894T, & 4b/a) and GST (M1 & T1) genes polymorphisms as well as Ca and vitamin D with the occurrence of myocardial infarction (MI) in Bangladeshi population. A case-control study on 100 cardiac patients with MI and 150 control subjects was conducted. The genotyping of ACE, NOS3 and GST genes were done using PCR and PCR-RFLP methods. Over all we found significantly ($p < 0.01$) higher level of troponin I in cardiac patients while the Ca and vitamin D levels were significantly ($p < 0.001$, respectively) lower in patients. The percentage of DD genotypes of ACE gene was significantly ($p < 0.05$) higher in patients. The individual with DD allele was at 3.2 fold risk {odds ratio (OR) = 3.28; 95 % confidence interval (95 % CI) = 1.6 to 6.7; $p < 0.01$ } of experiencing MI while individual with ID genotype was at lower risk. The cigarette smokers with DD genotypes were found to have a 4.1-fold increased risk to develop cardiac disease (OR = 4.1; 95 % CI = 1.7 to 10.5; $p < 0.01$). The frequencies of NOS3 and GST genotypes in the patients and controls were almost similar. Thus our recent study suggested that Ca and vitamin D deficiency, and ACE (I/D) gene may have strong associations with the occurrence of MI.

P-993

Single-molecule studies on CFP-YFP-based biosensorsH. Höfig^{1,2}, M. Pohl³, V. Steffen³, J. Otten³, A. Boersma⁴, J. Fitter^{1,2}¹I. Institute of Physics (IA), RWTH Aachen University, Germany; ²Institute of Complex Systems, Molecular Biophysics (ICS-5), FZ Jülich GmbH, Germany; ³Institute of Bio- and Geosciences, Biotechnology (IBG-1), FZ Jülich GmbH, Germany; ⁴Biomolecular Sciences and Biotechnology Institute, University of Groningen, Netherlands

Genetically encoded FRET-based biosensors consist of two fluorescent proteins (donor and acceptor) and a sensing domain. The ensemble readout usually utilizes the ratio of fluorescence emission of the donor and the acceptor. However, ensemble FRET measurements are biased by varying fractions of donor-only molecules. We carried out unbiased single-molecule measurements for two types of CFP-YFP biosensors; one is sensitive to glucose concentration [1] and another one monitors macromolecular crowding [2]. The obtained FRET efficiencies histograms dissect the different subpopulations of the sensors under varying environmental conditions. Additionally, we developed an improved two-color coincidence algorithm to quantify the fractions of donor-only, acceptor-only, and donor-acceptor molecules. In combination with time-resolved anisotropy measurements we aim to understand the sensing mechanisms and, thus, enable a rational sensor design [3].

[1] R. Moussa et al., *J. Biotechnol.* **2014**, 191, 250-259[2] A. J. Boersma et al., *Nat. Methods* **2015**, 12, 227-229[3] H. Höfig et al., **2017**, submitted

P-995

Effects of microwave radiation on dendritic spines and SNK-SPAR pathway in hippocampal neurons

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Epidemiological and experimental studies showed that microwave radiation could result in sleep disorder, abnormal EEG and deterioration of the memory. SNK-SPAR pathway plays a key role in synaptic reconstruction depending on activation of neurons. It is reported that the microwave radiation could induce abnormality in dendritic spines plasticity, however, the mechanism are rarely reported. In the study, we found that 30mW/cm² microwave radiation could result in the density of dendritic spines and percentage of mature spines decreased in cultured primary hippocampal neurons; the increased SNK which led to the decrease of SPAR and PSD-95; low expression of Calcineurin which induced the decrease of CREB, the increase and nuclear translocation of p-CREB, then lifted the transcription level of SNK; the phosphorylated modification site in GKBD, which is the specific binding structure domain with PSD-95 of SPAR, transformed from ser-1567 and ser-1472 to ser-1603, and the interaction of SPAR and MAP2. It might lead to obstacle in spine maturation and debilitate synaptic connections.

Key words: microwave; dendritic spines; SNK; SPAR

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P-996

Structural studies on the perireceptor proteins involving in the chemoreception

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As odorants are small hydrophobic molecules and chemoreceptors are covered with a layer of mucous or lymph fluid, it would be reasonable to expect a molecule which transports hydrophobic odorants to their receptors through the hydrophilic layer. Odorant-binding protein (OBP) and chemosensory protein (CSP), sometimes called as perireceptor proteins; PRP, found in the chemosensory organ, are water-soluble proteins that can bind to the hydrophobic small molecules. These two types of protein are expected to carry the hydrophobic small molecules up to the receptors. The molecular mechanisms of the process, however, are not well elucidated. Especially, the mechanism how the proteins release the odorants is a key issue to understand the physiological role of the perireceptor proteins. CpLip1, a lipocalin-type OBP found in the olfactory epithelium of *Cynops pyrrhogaster*, and CjapCSP1 found in the outer sensillum lymph of sensilla chaotic of the carpenter ant, *Camponotus japonicus* were investigated to elucidate the issue. We measured the CD and SWAXS spectra, and the fluorescence from an intrinsic tryptophan or that from a fluorescent dye bound to them at several different pH's. The results suggest that an environmental pH has significant effects on the structure and ligand-binding capacity of Cp-Lip1 and CjapCSP1.

P-998

Conformational dynamics of NTS1 helix 8

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G protein-coupled receptors (GPCRs) are the largest class of cell surface receptors encoded by the human genome, and are of considerable biological and pharmacological significance. They exhibit high levels of structural plasticity, which gives rise to poor thermostability outside of a membrane environment and hampers crystallisation efforts, necessitating the use of thermostabilising modifications. Crystal structures of thermostabilised neurotensin receptor 1 (NTS1) constructs are inconsistent on the presence and extent of the secondary structure element helix 8, an element implicated in G protein & arrestin activation for other GPCRs. Here, site-directed spin labelling and continuous-wave electron paramagnetic resonance (CW-EPR) are employed to analyse the dynamics and secondary structure of the putative NTS1 helix 8, making use of the non-thermostabilised receptor in a functionally supporting membrane environment of native-like lipid composition. The data reveal large effector-dependent changes in the dynamics of helix 8 and the C-terminus upon receptor activation, and indicate roles for the lipid environment in modulating C-terminal structure and GPCR-arrestin complex formation.

P-997

Serotoninergeric receptors (SR) as a target of TEMS

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TEMS is using for treatment of depression. Mechanism of effect is not understood. Hypothesis is TEMS activates of the SR of the brain.

The goal was to test possibility to activate SR via TEMS in proposed regime.

Experiments were based on influence of activation of SR on delay of the active avoidance conditional reflex (ACR) development.

33 rats were divided into 3 groups. ACR was developed in the group 1 after TEMS, in group 2 after TEMS and intraperitoneal injection of a SR antagonist "Kitril". The third – control.

In groups 1 & 2 TEMS was performed 10 min prior to start of development of ACR. The power density was 150 MW/cm².

Group 1- in 50% cases suppression of ACR (P<0.05) was noted, in 25% cases a complete inhibition of ACR (P<0.05) was achieved.

Group 2 - in 91% cases didn't noted (P>0.05) suppression of ACR versus control.

TEMS in groups 1 & 2 did not effect the process of retention of ACR when acquired (P>0.05) compared to the control.

The TEMS results in activation of SR. SR are targeted not only by TEMS, but also by a variety of pharmaceutical drugs: antidepressant, anti-migraine, antianxiety, sleep pills and some others. Activation SR via the TEMS in proposed regime could be used for treatment not only symptoms associated with depression but also with other conditions.

P-999

CalQuo2: automated, Fourier space quantifications of population-level global calcium responses

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High temporal resolution, fluorescent light microscopy is a vital tool in capturing live cell events that are both transient and dynamic. Calcium signalling is one such event that participates in a wide range of cellular life, including cellular immunity. However, analysing calcium signalling in a quantifiable, automatic manner of single, non-excitable cells within a large sample is challenging. By automatically segmenting images and quantifying the fraction of cells that flux calcium, the software, *CalQuo*, offers some automatic features quantifying calcium responses. *CalQuo*² expands upon these automatic features by reducing the number of user-defined parameters, while also providing a fully automated option in determining whether a cell has fluxed calcium. Additionally, *CalQuo*² can distinguish between single and oscillatory calcium fluctuations. We have demonstrated the use of *CalQuo*² by measuring the calcium responses in genetically modified T cells exposed to varying ligand conditions, in which we have found that peptide-MHCs and anti-CD3 antibodies trigger a fraction of T cells to release oscillatory calcium fluxes that increase with increasing k_{off} rates.

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– 32. Receptors and signalling –

P-1000

On biophysics of interaction hormones - cell receptors on example of blood pressure

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Introduction: Nicotine/N, hormones: acetylchol./ACH, 5-HT, vasopr./VP have very *complex effects* on blood circulation (*Harvey* 1628) & neuro-regulation (Sir H.*Dale*, O.*Löwi*, J.*Axelrod*). Method: Blood pressure/BP of normal/spinal rats (n=120) (Strahlenther. 147:290-7/1976). Results (recent/earlier). MEG effects (2-mercaptoethylguanidine), inhibitor of Ca-indep. isoform of NO-synthase (10-30mg/100g): [1] MEG&N changed depressor-reaction (dR) to ACH (0.1-1µg/100g) into biphasic (dR/pR=pressor) one by nicotinic cholinergic receptors (=nACHRs), also dR to electrical central vagal stimulation (55Hz, 2ms, 5s, 5V). [2] Potentiation of BP-reactions to peptide hormones: MEG potentiates VP pR (octapeptide: 0.05-5mU/100g) & bradykinine (nonapeptide) dR. [3] Inversion of 5-HT (0.1-6µg/100g) dR into pR by MEG. Conclusion: Clarification of interaction MEG-N - chemical structure of CNS-types from nACHRs (esp. alpha-4₂-beta₂₃), neuronal VPR1b & vascular VPR1a receptors, 5-HT1-7 receptors (G-protein coupled), esp. 5-HT1a-f, 5-HT2a-c, 5-HT7, related to new MEG-derivatives could open new therapy-approaches of art. hypertension, cardiac arrhythmia, tobacco pathology.

P-1002

Spatial relationship of IL-9 and IL-2 receptors at the surface of human T lymphoma cells

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The heterodimeric IL-9R consists of the cytokine-specific α subunit and the common γ_c chain. Expression of IL-9R α is mainly restricted to T cell subsets expressing the heterotrimeric IL-2R as well, where IL-2 has a crucial regulatory role. The two receptor kinds share the signaling γ_c chain, therefore the question arises what their spatial relationship is and how it is affected by the cytokine milieu. Our confocal microscopy co-localization and FRET experiments demonstrated the bipartite spatial relationship of IL-9R with IL-2R in human T lymphoma cells: in addition to their co-expression in common membrane domains, a considerable fraction of IL-9R and IL-2R could also be detected in spatially segregated membrane areas. The presence of distinct IL-9R domains is not mutually exclusive; they may co-exist on the same cell. IL-2 deprivation reduced slightly the overlapping fraction of the receptors, implying that IL-2 may have a regulatory role in the cell surface organization of IL-9R. Short term exposure to IL-9 did not cause significant rearrangement of the receptors at the few-hundred-nanometer scale, but changed FRET efficiencies between the subunits of the IL-9/2R system, i.e. IL-9 binding either alters the conformation of the IL-9/2R complex or causes lateral redistribution of the receptor chains on the nanometer scale.

P-1001

The dynamics of onset of AHL-mediated quorum sensing in *Pseudomonas aeruginosa*

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Inter- and intraspecies communication has a special role in the life of a microbial community. Thus the presence/absence of signal molecules has a great impact on a bacterial population. Quorum sensing (QS) is a fundamental type of communication that allows microbial populations to perform synchronised group behaviour that happens through simultaneous production and detection of specific signal molecules (HSL). Such system offers several advantages to communities, e.g. production of virulence factors and antibiotics, biofilm formation. We investigate the dynamics of onset of the quorum state in *P. aeruginosa*. We use microfluidic approaches to study how QS “turns on” at cellular level and to reveal details of the relation between QS and bacterial motility. Mutants containing fluorescence reporter plasmid are used to detect the changes in the QS state upon adding exogenous HSL. The effect of HSL concentration, nutrient supply and flow rate of media has been tested in our system. We found that the fluorescence intensity (corresponding to the QS state) of the cells reaches a maximum value within 1-2 hours, and it remains high for several hours after washing the sample with pure HSL-free media. The cell-to-cell variability in the fluorescence intensities was also analyzed.

P-1003

The role of the actin cytoskeleton in regulating receptor dynamics and function

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The dynamic states of membrane receptors modulate the mechanisms of ligand interaction and can indicate the functional properties of the receptor. There are many molecular players involved in contributing to these events. One such contributor is the cortical actin cytoskeleton. In this study, we investigate the role of the actin cytoskeleton in regulating the dynamics and function of a lymphatic endothelial receptor, LYVE-1. Using super-resolution STED microscopy we quantified the clustered states of LYVE-1 that are known to cause avidity induced receptor function. We also identified a freely diffusing and an immobile state of LYVE-1 using FRAP which indicates the involvement of the cytoskeleton in LYVE-1 dynamics. Hence to look at the heterogeneous diffusion population of LYVE-1, we used scanning FCS (sFCS) on primary and transfected lymphatic endothelial cells in actin depleted conditions. Our sFCS results suggest that LYVE-1 mobility decreases after actin depletion and correlates with an increase in ligand interaction as determined by FACS. This data along with biochemical assays not only show a direct link of the actin cytoskeleton with LYVE-1 function and dynamics, but also highlights the necessity of employing a range of complementary techniques to decipher molecular functions in cells.

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– 32. Receptors and signalling –

P-1004

Pathogenic mechanism of the W64R mutation in human β_3 -adrenergic receptor as studied by confocal fluorescence microscopyC. Sun¹, X.-Y. Ding¹, Y.-N. Yang¹, A. Watts², X. Zhao¹¹East China Normal University, China; ²University of Oxford, UK

β_3 -adrenergic receptor (β_3 -AR), a rhodopsin like GPCR locating mainly in adipose tissue, involves in the regulation of lipolysis and thermogenesis. W64R, a common mutation in human β_3 -AR, is associated with many diseases including obesity, type 2 diabetes and various related diseases. However, little is to know about the pathogenic mechanism of this W64R due to the lack of necessary information at the cellular and molecular level. In this study, we successfully integrate the enhanced Green Fluorescent Protein (eGFP) as a fusion tag with the wild type or the W64R mutated human β_3 -AR to form a fusion protein. It is then expressed in the HEK293S cell lines, and the fluorescent tracers are analyzed through confocal fluorescence microscopy. Distribution, stability and insertion efficiency into the membrane of the wild type and W64R mutant are carefully examined. Our results show that most of the W64R mutants still remain in the cells to form aggregates, resulting in a lower insertion efficiency than that of the wild type, although part of the mutant protein could arrive successfully at the membrane. Blocking the inserting process of β_3 -AR into the cell membrane might be a possible pathogenic mechanism, since a lower stability and easier aggregation state of the mutation may cause function loss of β_3 -AR, which might eventually lead to various diseases.

P-1006

A new perspective in class A GPCRs: The interplay between membrane voltage, cations and protonsO. N. Vickery¹, C. A. Carvalheda¹, S. A. Zaidi², A. V. Pislakov¹, V. Katritch², U. Zachariae¹¹Dundee University, UK; ²University of Southern California, USA

G-protein coupled receptors (GPCR) are the largest superfamily of transmembrane proteins. Due to their central role in transmembrane signal transduction, GPCRs represent 30% of the druggable genome and 25% of the targets of commercialized drugs. However, how ligand binding triggers signal propagation through GPCRs, and subsequent activation of intracellular effector proteins, remains poorly understood. High-resolution crystal structures have recently revealed that class-A GPCRs harbour a Na^+ ion at the base of an aqueous pocket in the inactive state. The ion is bound to a fully conserved residue, D^{2.50}, which is accessible only from the extracellular solution in the crystal structures. In their physiological environment in the cell membrane, GPCRs experience steep electrochemical gradients as well as the membrane voltage (V_m), which creates a strong electric field of the order of 10^8 V/m within the lipid bilayer.

We used molecular dynamics and computational electrophysiology simulations to study the interplay between V_m , internal ion occupation and protonation of GPCRs. Our results reveal a potential mechanistic underpinning for the experimentally observed voltage sensitivity of GPCRs, which is of great relevance for GPCR drug design in electrically excitable cells such as neurons.

P-1005

Novel fluorescent probes for retinoic acid binding proteinsC. W. Tomlinson¹, D. R. Chisholm¹, R. Valentine³, A. Whiting¹, E. Pohl²¹Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK; ²Department of Biosciences & Biophysical Sciences Institute, Durham University, South Road, Durham DH1 3LE, UK; ³High Force Research Ltd., Bowburn North Industrial Estate, Bowburn, Durham, DH6 5PF, UK

Development of new, fluorescent, retinoic acid analogues offers the opportunity to investigate retinoic acid binding proteins both in vitro and in vivo. Retinoic acid signaling is vital for normal growth and development, and offers an attractive target for novel therapies, particularly within the field of neurodegenerative disease. Through different binding affinities the capability of, and variation between, different protein isoforms can be investigated, and potentially harnessed to design isoform-specific ligands. The fluorescent nature of the probes, and their enhanced stability over that of retinoic acid, makes them ideal for following in vitro via fluorescence spectroscopy and will be invaluable for high throughput ligand screening - feeding into the structure based design of future ligands.

P-1007

Assembly of interleukin receptor subunits during traffickingJ. Volkó¹, Á. Kenesei¹, P. Várnai², F. Bestvater³, T. A. Waldmann⁴, K. Tóth³, G. Vámosi¹¹Department of Biophysics and Cell Biology, University of Debrecen, Hungary; ²Department of Physiology, Semmelweis University, Budapest, Hungary; ³German Cancer Research Center, Heidelberg, Germany; ⁴Lymphoid Malignancies Branch, CCR, NIH, Bethesda, USA

Multisubunit receptors of interleukin-2 and -15 cytokines may exist in the membrane of immune cells as heterodimers or -trimers having different ligand binding affinities. Pre-assembly of the subunits has already been characterized at the cell surface but their formation inside the cell after their synthesis remains to be clarified. Our aim was to investigate the associations of subunits in the ER and Golgi.

We created plasmid constructs expressing different receptor chains tagged with EGFP or mCherry. After co-transfection in HeLa cells, intensity based Förster Resonance Energy Transfer was measured between the subunits by confocal microscopy. TagBFP-tagged ER and Golgi markers were used to localize these organelles, FRET data was analysed in an organelle specific manner using FiJi. IL-2/15R subunits showed positive FRET efficiency during their trafficking. Data suggest that the subunits associate prior to reaching the cell membrane.

In IL-2 producing T cells signaling might take place in the cell before receptor chains are expressed in the plasma membrane. Our results may have clinical importance in antibody therapies against lymphoma targeting receptor subunits.

Posters**– 32. Receptors and signalling –****P-1008****MiR-21 contributes to bystander effects through exosome-mediated microRNA transfer**

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Bystander effects are well-established phenomena which results in damage of non-irradiated cells in response to signals transmitted from irradiated cells. The signals can be cytokines, reactive oxygen species, nitric oxide and small non-coding RNAs. Recently, we found that miR-21 was involved in bystander effects mediated by condition medium through exosome. MiR-21 was significant and consistent up-regulated in both directly irradiated cells and bystander cells. Overexpression of miR-21 in non-irradiated cells could induce bystander effects such as high frequency of micronuclei, high yields of 53BP1 foci and low survival fraction. Inhibition of miR-21 could offset the bystander effects to some extent. It was also found that transfection of exosomes isolated from conditioned medium into non-irradiated cells could induce bystander effects. Fluorescence labeled exosomal miR-21, which was up-regulated as a result of mimic transfection or irradiation, could be transferred from the donor (irradiated cells) into extracellular medium and subsequently got access to the recipient (bystander cells) to induce bystander effects. These findings provide new insights into the functions of microRNAs and the cellular communication between the directly irradiated cells and the non-irradiated cells.

P-1010**Real-time probing the spatiotemporal interaction between binding of TNF- α to TNF-receptor and the corresponding NF- κ B signaling dynamics by a single-cell approach**

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Tumor necrosis factor (TNF- α) is an inflammatory cytokine produced mainly by macrophages. Binding of TNF- α to TNF receptor (TNFR) activates nuclear factor kappa B (NF- κ B) transcription factors, leading to oscillations in nuclear NF- κ B and target gene expression by negative feedback loops. However, direct observation of the spatiotemporal interaction between TNF- α stimulation and the corresponding NF- κ B signaling dynamics has remained elusive. We have developed a platform in which the temperature-controlled microfluidic flow cell is incorporated into fluorescence microscopy systems. In addition, we utilize the biofunctionalized quantum dots conjugated with TNF- α to monitor the cellular distribution of TNF- α , which can provide a direct access to probing the spatiotemporal distribution of TNF- α -TNFR complex. On the other hand, the intracellular p65 was labeled with RFP for NF- κ B tracking. The observation demonstrated that antibody-functionalized quantum dots are more adequate for live monitoring the binding of TNF- α to TNFR in living NIH-3T3 cells. Furthermore, we also established an optimized method for labeling TNF- α , it can help us to explore its signal transduction pathway and the spatiotemporal interaction between binding of TNF- α to TNFR and the corresponding NF- κ B dynamics.

P-1009**Circulating microRNAs responding to ionizing radiation alleviate radiation damage to immune system**

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Recent studies show that specific circulating microRNAs (miRNAs) in blood are response to ionizing radiation, but the fate and function of those circulating miRNAs is still unclear. We performed miRNA PCR array to analyze the miRNA expression profiles obtained from the blood of total body irradiated mice after exposure to carbon ions and X-rays. Five miRNAs (miR-21, miR-34a, miR-200b, miR-342 and miR-574) differently expressed after exposure. Among them, miR-574 had the most obvious decline trend. Gene Ontology analysis revealed that miR-574 may involve in immune system process. Then, we up-regulated the level of miR-574 in blood of mice by tail injection of agomiRNA after exposure to X-rays. At three days and seven days after irradiation, the lymphocyte population was detected by flow cytometry. Compared with irradiation group without injection of miR-574's agomiRNA, injection groups had higher counts in T cells, B cells and Natural Killer cells. In this study, we have identified a set of serum miRNA which respond to ionizing radiation, and those miRNAs have potential of serving as noninvasive biomarkers for radiation risk assessment. In addition, some specific circulating miRNAs can alleviate radiation damage to immune system.

Posters

– 33. Membrane-active peptides –

P-1011 (O-198)

Single-molecule microscopy of Staphylococcal pore-forming toxins on live mammalian cells

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The majority of Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria produce pore forming toxins, specifically targeting white blood cells (leucocytes), which help infecting cells avoid immune response. Pantone-Valentine Leukocidin is one of these toxins and comprises two protein subunits, LukS and LukF, which interact with the human C5a receptor (hC5aR) to form holes in the cell membrane and kill the cell. We have labelled LukS/F and hC5aR with different fluorophores and imaged their interaction in live mammalian cells using rapid single-molecule total internal reflection fluorescence (TIRF) microscopy. We find LukS binds first to hC5aR and forms tetramers within hC5aR clusters. LukF is recruited to these LukS-hC5aR complexes leading to lytic pore formation and simultaneous dissociation of LukS-LukF from hC5aR. Our findings support a crystallographic heterooctamer model but provide a new view on the kinetics of these crucial virulence factors and their interactions with live cells.

P-1013 (O-196)

Single-molecule visualization of dynamic transitions of pore-forming peptides

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Research on the dynamics of single-membrane proteins remains underdeveloped due to the lack of proper approaches that can probe in real time the protein's insertion depth in lipid bilayers. Here we report a single-molecule visualization method to track both vertical insertion and lateral diffusion of membrane proteins in supported lipid bilayers by exploiting the surface-induced fluorescence attenuation (SIFA) of fluorophores. The attenuation follows a d^{-4} dependency, where d is the fluorophore-to-surface distance. The method is validated by observing the antimicrobial peptide LL-37 to transfer among five transmembrane positions: the surface, the upper leaflet, the centre, the lower leaflet and the bottom of the lipid bilayer. These results demonstrate the power of SIFA to study protein-membrane interactions and provide unprecedented in-depth understanding of molecular mechanisms of the insertion and translocation of membrane proteins.

P-1012 (O-197)

Beta amyloids aggregation at the surface of model functional membrane domains

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A hallmark pathological feature of Alzheimer's disease (AD) is the aggregation and deposition of β -Amyloid peptides (A β) in the brain. The interaction of different A β peptides with model membranes with biomimetic composition, mimicking functional domains containing phospholipids, cholesterol and glycolipids, has been studied by different complementary techniques. Calorimetry and X-ray and neutron scattering have been applied to investigate the thermotropic and structural behavior of membranes in bulk, while neutron reflectivity has been applied to study, with the Angstrom sensitivity, the structural interaction of peptides with asymmetric complex membranes prepared by the Langmuir-Blodgett deposition technique. Peptide-membrane interaction was found to depend both on membrane composition and on the state of aggregation of the peptide. Moreover the N-terminal portion of A β was seen to interact with lipids of the bilayer probably promoting the penetration of the peptide in the membrane. Rondelli et al., *Amyloid- β Peptides in interaction with raft-mimic model membranes: a neutron reflectivity insight* Sci. Rep. 6:20997 (2016)

P-1014

The number of α -synuclein proteins per vesicle gives insights into its physiological function

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Although it is well established that the protein α -synuclein (α S) plays an important role in Parkinson's disease, its physiological function remains largely unknown. α S has been reported to bind membranes and to play a role in membrane remodeling processes. The mechanism by which α S remodels membranes is still debated; it may either affect its physical properties or act as a chaperone for other membrane associated proteins.

To obtain insight into the role of α S in membrane remodeling, we investigated the number of α S proteins associated with single small vesicles in a neuronal cell model expressing α S-GFP. Using single-molecule microscopy and photo-bleaching approaches, we found the number of α S-GFP distributed around 70 per vesicle. This number is high enough to modulate physical membrane properties. Yet it is also strikingly similar to the number of synaptobrevin, a putative interaction partner of α S, per vesicle. We therefore hypothesize a dual synergistic role for α S in membrane remodeling acting both as a modulator of the membrane's physical properties and interaction partner for synaptobrevin.

Posters

– 33. Membrane-active peptides –

P-1015

Packing of peptides on the surface of lipid membranes

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Despite of the great impact of surface chemistry in technical chemistry and surface physics in nanotechnology the role of lipid membranes as surfaces with very specific properties was neglected for long-time.

The fluid mosaic model of lipid membranes of Singer and Nicolson [Singer S.J. and Nicolson G.L. Science (1972) 175, p720] dominated our picture of lipid membranes as two dimensional liquids. However at high concentration the finite size of peptides and proteins incorporated into lipid membranes lead to crowding effects. The influence of the crowding effects at high peptide concentration on the adsorption isotherms was investigated theoretically and experimentally in few publications.

Here we extend the experiments to a microscopic picture, by focusing on the distance between peptides at different conformation as measured by fluorescence self quenching. We are able to describe the effect of size and conformation on the packing of peptides on lipid surfaces. Furthermore our data suggests the presence of a nematic phase made of two dimensional rods representing the in-planar peptides. The data opens a window to describe the surface chemistry and nanotechnology involved in biochemistry.

P-1017

Surface acoustic waves-based molecular recognition of a collagen receptor on human erythrocyte ghostN. Ayvazyan¹, N. Ghazaryan¹, T. Gronewold¹

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Snake venom disintegrins obtustatin and echistatin are potent, irreversible and selective inhibitors of $\alpha 1\beta 1$ and $\alpha v\beta 3$ integrins respectively. Aiming to describe the structural requirements of disintegrins for membrane-target recognition, the affinity of these specific binding have to be elucidated, being this topic an issue of extremely importance for the human health. Obtustatin is the shortest disintegrin yet described, containing only 41 amino acids. It contains a similar pattern of cysteines to the short disintegrin echistatin but contains the sequence KTS rather than RGD in its active site loop. To confirm molecular recognition of disintegrins by their substrates, a surface acoustic wave-biosensor was applied. The human erythrocyte ghosts (EG) were immobilized at the sensors to detect of kinetic binding constants of disintegrins compared to GUVs surface. Obtustatin binds to EG membrane with affinity in mid-nanomolar range (2.32×10^{-7} M), and echistatin - in the micromolar range, which indicates specific molecular recognition for disintegrins with higher response for obtustatin. The data provide evidence for a direct confirmation of disintegrin binding to EG membrane and thus, contribute to prove the presence of integrins in the red cell membranes earlier neglected.

P-1016

Broad spectrum antiviral activity modulated by biophysical properties of fusion inhibitory peptidesM. T. Augusto¹, C. Mathieu², M. Porotto³, A. Moscona^{3,4}, N. C. Santos¹

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Human paramyxoviruses cause human respiratory diseases with a significant focus in infants and children. This viral class comprises the causing source of common croup – parainfluenza viruses – as well as agents of lethal encephalitis, like Nipah virus. Infection is initiated by viral glycoprotein-mediated fusion between viral and host cell membranes. Paramyxovirus viral fusion proteins (F) insert into the target cell membrane, and form a transient intermediate that pulls the viral and cell membranes together as two heptad-repeat regions refold to form a six-helix bundle structure that can be specifically targeted by fusion-inhibitory peptides. Antiviral potency can be improved by sequence modification and lipid conjugation, and by adding linkers between the peptide and lipid components. We investigated the membrane insertion kinetics and cell membrane affinity of broad spectrum parainfluenza F-derived peptides that inhibit both parainfluenza and Nipah viruses. Fluorescence-based techniques were used to exploit peptides-membrane interaction. The engineering approach based on biophysical parameters resulted in a peptide that is a highly effective inhibitor of both paramyxoviruses and a set of criteria to be used for engineering broad spectrum antivirals for emerging paramyxoviruses.

P-1018

Towards a nanoscale description of the interactions between the peptide A β (1-42) with membranesM. Azouz^{1,2}, C. Cullin¹, M. Lafleur², S. Lecomte¹

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Alzheimer's disease is considered as the major form of dementia, with an estimation of 130 million of people living with the pathology by 2050, worldwide. In the project, we aim at determining the interactions between Alzheimer's neurotoxic peptide A β ₁₋₄₂ with some lipid components of neuron membranes. We focus our study on the effects of cholesterol and GM1 on the interaction with the peptide. Polarized Attenuated Total Reflection Infrared Spectroscopy (pATR-FTIR) allowed us to observe the impact of the membrane composition on the interaction while high speed AFM in aqueous condition is used to define the evolution of the lipid bilayer morphology upon the addition of the peptide. Key information about the kinetics of the bilayer fragmentation, peptide auto-assembly, and domain formation were collected. Composition dependant lipid bilayer extraction was observed and quantified as ν_{CH_2} band intensity, observed by pATR-FTIR, decreased after the peptide was introduced. Small amounts (10 mol%) of cholesterol, or GM1 in POPC membranes led to significant disruptions unlike pure POPC bilayers where no effect was observed. These results highlight the pivotal role of membrane composition in A β ₁₋₄₂ effect.

Posters

– 33. Membrane-active peptides –

P-1019

Identification of molecular scale changes in model membranes due to lipid – protein interactionsI. Brand¹, K.-W. Koch²¹University of Oldenburg, Department of Chemistry, Germany; ²University of Oldenburg, Department of Medicine, Germany

Lipid-protein interactions influence the macroscopic and microscopic properties of membranes such as the permeability to ions, capacitance, lateral composition, orientation and physical state of lipid molecules as well as the activity and structure of proteins. Static electric fields in the order of $10^7 - 10^9 \text{ V m}^{-1}$ constitute to the natural environment of lipids and proteins present in biological membranes.

In situ studies of changes in biological membranes due to the lipid-protein interactions exposed to physiological electric fields bring a need of use bioanalytical methods which would allow measurements of the composition and function of these complex biological systems. A reflection-based IRS technique: polarization modulation infrared reflection-absorption spectroscopy is an excellent analytical tool to study electric potential-driven changes in the structure of lipid membranes at a sub molecular level. In this study changes in the orientation, physical state and hydration of lipid molecules in asymmetric models of membranes during their interactions with proteins under physiological electric fields are reported. Simultaneously the structure of proteins: Ca^{2+} dependent recoverin and sigelc interacting with model membranes is discussed.

P-1021

MPER-derived virucidal peptides disrupt the HIV-1 lipid envelope functional organizationP. Carravilla¹, A. Cruz², I. R. Oar-Arteta¹, J. Pérez-Gil², J. Requejo-Isidro¹, N. Huarte¹, J. L. Nieva¹¹Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), Spain; ²Department of Biochemistry, Faculty of Biology, and Research Institute Hospital 12 de Octubre, Universidad Complutense, Spain

HIV-1 is an enveloped virus surrounded by a cholesterol-enriched lipid membrane. The envelope glycoprotein transmembrane subunit gp41 fuses the viral envelope and the cell plasma membrane after receptor and coreceptor recognition by the gp120 subunit. The membrane proximal external region (MPER) and transmembrane domain (TMD) of gp41 restructure the viral bilayer during the fusion process. Our data suggest that the HIV-1 lipids are tightly-packed and, unexpectedly, separated laterally into nanodomains and that viral infectivity strongly depends on these two features. Moreover, virus exposure to a MPER-TMD-derived peptide before the fusion process is activated inhibits viral infection. This peptide is also able to alter the phase-organization of the lipid envelope, thereby suggesting that lipid nanodomains play a key role during HIV-1 infection. We believe that these results can contribute to the understanding of the role of the MPER domain during fusion and also help in the design of novel antiviral compounds targeting the HIV-1 envelope.

P-1020

Interactions of misfolded proteins with lipid membranes: Implications for neurodegenerationR. Budvytyte¹, P. Cizas², M. Lösche³, G. Valincius¹¹Life Sciences center, University of Vilnius, Lithuania;²Neuroscience institute, Lithuanian University of Health Sciences, Lithuania; ³Dept. of Physics, Carnegie Mellon University, USA

A central event in pathogenesis of neurodegenerative diseases are thought to be intracellular and extracellular accumulation, aggregation and misfolding of low molecular mass peptides[1] such as β -amyloid (in Alzheimer's), α -synuclein (in Parkinson's) and others. Small size aggregates-oligomers were found to be extremely neurotoxic *in vitro* and *in vivo* with the ability to disrupt the major neuron membranes[2] and lead to synaptic dysfunction, mitochondrial dysfunction[3], neuronal apoptosis and brain damage[4]. In this work different sizes of soluble recombinant $\text{A}\beta_{1-42}$ and α -syn aggregates were used to investigate their interaction with tethered phospholipid membranes (tBLM) as well as their toxicity to rat cerebellar granule cells (CGC)[5]. The morphology and size of misfolded protein aggregates was monitored by DLS and AFM. However, differently sized amyloid oligomers exhibited different levels of neurotoxicity in CGC toxicity tests. These protein aggregates exhibited the membrane damaging properties as probed by the electrochemical impedance spectroscopy (EIS). Amyloid induced membrane conductance exhibited relatively weak temperature dependence, which is similar to water-filled pores formed by pore forming toxins. Membrane composition was found to be one of the important factors affecting the interaction of the $\text{A}\beta_{1-42}$ oligomers to phospholipid membranes.

P-1022

Characterization of the interaction between a small dicationic peptide and lipid membranesP. Chervy^{1,2}, D. Rault^{3,2}, C. Chassaing², J. Richard², F. Artzner³, M. Paternostre¹¹I2BC/B3S/IMAPP, Gif-sur-Yvette, France; ²Ipsen, France; ³IPR, France

Molecular self-assembly plays an important role in many biological systems but the physicochemical interactions which drive these processes are still unclear. For example, the interaction between amyloid peptides and membranes is believed to be a key step in amyloidogenesis. In this work, we used a small di-cationic synthetic self-assembling peptide as a model compound to evaluate interactions with lipid membrane.

Biological membranes are globally negatively charged. Therefore, we used two phospholipids the phosphatidylcholine which is a zwitterion and phosphatidic acid which is anionic. By varying the proportion of the anionic lipid we generated membranes with increasing charge density and we added different quantity of peptide in order to obtain different charge ratio between the two positive charges coming from the peptide and the negative ones coming from the lipids. The samples were structurally characterized by X-Ray scattering, negative staining and freeze fracture electron microscopy and infrared spectroscopy.

This phase diagram approach allowed us to determine the structure of the lipid-peptide assemblies. We also determined different interaction intermediates. By varying lipid composition, we show that this peptide-lipid architecture is robust provided we keep anionic lipid in the mixture.

Posters

– 33. Membrane-active peptides –

P-1023

The tumor homing peptide tLyp shows penetrating properties in model membranesC. Ciobanasi¹, A. Apetrei²¹Department of Interdisciplinary Research, Alexandru I. Cuza University, Iasi, Romania; ²Department of Physics, Alexandru I. Cuza University, Iasi, Romania

Cell penetrating peptides (CPPs) have the property to cross lipid bilayers and were successfully used to deliver various macromolecular cargoes such as imaging and therapeutic agents, gold nanoparticles, DNA and even liposomes. The exact molecular mechanisms of CPPs translocation across membranes are still under debate and vary from passive to active mechanisms. The tLyp peptide is a truncated form of the cyclic tumor-homing peptide LyP-1. It is similar to CPPs but it binds specifically to neuropilin receptors which are overexpressed in glioma tumor tissue. Unlike CPPs, the cellular uptake of the homing peptides appears to be an energy-dependent process.

We use a well-defined model membranes, giant unilamellar vesicles (GUVs) to study the interaction between tLyp peptide and lipid membranes. Generally, model membranes are used to elucidate the non-endocytic entry routes of peptides because the energy-dependent processes are abolished. Our experimental results show that tLyp translocates GUVs membranes of different lipid composition, suggesting that the internalization of this peptide also involves a passive, energy-independent mechanism of uptake.

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P-1025

Antimicrobial peptides PaMAP 1.9 and 2 are efficient against a clinical multi-resistant bacteriaM. R. Felício¹, O. L. Franco^{2,3}, N. C. Santos¹, S. Gonçalves¹

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Some pathogens have become multi-resistant to the current available drugs, inducing persistent infections on patients. This paradigm led to the urgency on the development of new drugs. Antimicrobial peptides (AMPs) are pointed out as potential alternatives. This class of small, amphipathic and cationic peptides have been extensively studied in terms of peptide-membrane interactions, but this urgency has redirect to pharmacodynamics and effectiveness studies.

This work is focused in two AMPs (PaMAP 1.9 and 2) that have been synthetically designed from a natural AMP. With the promising initial results, peptide-membrane interactions were studied using lipid vesicles and bacteria. As bacterial strains, *wt* and a clinical multi-resistant strains (KPC) of *Escherichia coli* were used. Different biophysical techniques, including fluorescence spectroscopy, fluorescence microscopy, flow cytometry and circular dichroism, allowed to infer about the efficiency in bacterial eradication. Both AMPs showed different mechanisms of action against bacteria, despite the strain, supporting future studies to identify the pathways differently regulated upon peptide treatment.

P-1024

FGF13 maintains the noxious heat-evoked action potential and increases Na_v1.7 current densityF. Dong¹, L. Yang¹, L. Bao², X. Zhang¹

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Abstract: Objective Noxious heat causes pain by activating the peripheral terminals of small-diameter dorsal root ganglion (DRG) neurons. Our previous behavioral data showed that loss of FGF13, a non-secretory fibroblast growth factor, in small DRG neurons could selectively abolish noxious heat sensation. However, the underlying mechanisms are still not being revealed. The voltage-gated sodium (Na_v) channels are critical for the generation of action potentials (APs) in DRG neurons. In this study, we investigated whether FGF13 affected noxious heat-evoked AP and then identified the involvement of Na_v1.7. **Methods** Whole-cell patch clamp recordings were performed on small DRG neurons or HEK293 cells transfected with indicated plasmids to examine the function of FGF13 under noxious heat (>43°C) and the involvement of Na_v1.7. **Results** 1) Noxious heat-induced AP firing is reduced and can be rescued with administration of exogenous FGF13 by GST-FGF13B-TAT in FGF13-deficient DRG neurons. 2) FGF13 increases Na_v1.7 current density. 3) Disrupting FGF13/Na_v1.7 interaction by Na_v1.7CT-TAT attenuates AP firing following the heat stimulation. **Conclusion** The interaction between FGF13 and Na_v1.7 complex is essential for sustaining the transmission of noxious heat signals.

P-1026

Combining 25-hydroxycholesterol with a fusion inhibitor peptide: interaction with model biomembranes and human blood cellsB. Gomes¹, A. Hollmann², A. Moscona^{3,4}, M. Porotto³, N. C. Santos¹

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Human immunodeficiency virus type 1 (HIV-1) entry into target cells is a multi-step process, leading to the fusion of viral and cell membranes and the release of virus' content into the host cell. Enfuvirtide is a fusion inhibitor peptide clinically approved for HIV, but its use is associated with the development of viral resistance. Other fusion inhibitor peptides, as C34, were synthesized in order to overcome this limitation. In this work, C34 was conjugated with 25-hydroxycholesterol (25HC), an oxidized cholesterol derivative which inhibits HIV-1 entrance at the membrane level. Combining two distinct antiviral molecules may help to suppress the emergence of resistant viruses. We characterized the interaction of C34-25HC conjugate with biomembrane model systems (lipid vesicles and monolayers) and human blood cells. C34-25HC interacts preferentially with membranes rich in sphingomyelin and presents a poor partition to membranes composed of phosphatidylcholine and cholesterol. We hypothesize that cholesterol causes a repulsive effect, overcome in the presence of sphingomyelin. Importantly, our data indicates that the peptide has a preference for human peripheral blood mononuclear cells relative to erythrocytes, demonstrating its selectivity and potential to target CD4⁺ cells.

Posters

– 33. Membrane-active peptides –

P-1027

The ionization properties of histidine in lipid bilayers

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The ionization properties of histidine in lipid bilayer membranes help govern the functioning of many membrane proteins. The α -helical transmembrane peptide GWALP23 (acetyl-GGALWLALALALALWLAGE-ethanolamide) was used to address these properties. His residues were inserted at position 12 or 14 and ^2H -Ala labels were incorporated within the core sequence. Solid-state ^2H NMR spectra of GWALP23-H12 reveal pH dependent differences in peptide behavior. At neutral pH, GWALP23-H12 and GWALP23-H14 exhibit well-defined tilted transmembrane orientations in bilayer membranes. Under acidic conditions GWALP23-H12 exhibits a major population that moves to the bilayer surface and a minor population that occupies multiple transmembrane states, similar to GWALP23-R12 at neutral pH (JACS 132, 5803). The dramatic behavioral change of GWALP23-H12 suggests a pK_a of less than 3 to yield a neutral His imidazole of 3-5, to transition between orientations resembling GWALP23-K14⁺ at low pH and GWALP23-K14⁰ at high pH. Cholesterol modulates the multi-state behavior of GWALP23-R12, favoring a major interfacial state (Biochemistry 55, 6337).

P-1029

Efficient and reliable free-energy calculations of pore formation reveal a metastable prepore state

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The free-energy landscape of membrane poration remains poorly understood, mainly because reliable and efficient free-energy calculations of pore formation using molecular dynamics (MD) simulations have remained challenging. We present a new reaction coordinate (RC) for pore formation that probes the formation and rupture of a continuous polar defect over the membrane. Potential of mean force (PMF) calculations along the new RC rapidly converge and exhibit no hysteresis between pore-opening and pore-closing pathways, in contrast to calculations based on previous RCs. Remarkably, we observe that the PMF of pore formation in a tension-free DMPC membrane reveals a free-energy barrier for pore nucleation, confirming a long-hypothesized metastable prepore state.

[1] Hub and Awasthi, Probing a continuous polar defect: A reaction coordinate for pore formation in lipid membranes, *submitted*

[2] Awasthi and Hub, *J Chem Theory Comput*, 12, 3261–3269 (2016)

P-1028

Membrane binding of betinoldehydrogenase 8 (RDH8) and of its C-terminal segment

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Retinol dehydrogenase 8 (RDH8) is a 312 amino acid (aa) long protein involved in the visual cycle. Bound to the outer segments of photoreceptors it reduces all-trans-retinal to all-trans-retinol¹ as one of the rate-limiting steps of the visual cycle².

RDH8 is a member of the short chain dehydrogenase/reductase family. Its C-terminal segment allows its membrane-anchoring through the presence of 1 to 3 acyl groups³. The secondary structure and membrane binding characteristics of RDH8 and its C-terminal segment have not yet been described.

To evaluate the membrane binding of RDH8, the full-length protein, a truncated form, its C-terminal segment (aa 281–312) and 2 variants of this segment (W289F and W310R) were used. Tryptophan fluorescence spectroscopy was used to determine the extent of binding and to monitor its local environment. The partition coefficient of the peptides was determined to $K_p = 1.1 - 1.7^6$ independent of the type of lipid and presence of a mutation. The peptides secondary structure shows a superposition of α -helical, β -turn and unordered structures. The truncated protein binds membranes less efficiently than its full length form.

1Rattner et al. 2000, *J Biol Chem*, 275, 11034

2Saari et al. 1998, *Vision Res.* 38, 1325

3Lhor and Salesse 2014, *Biochem Cell Biol*, 92, 510

P-1030

Effect of mechanical property of membrane on entry of cell-penetrating peptides into single vesicles

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* The translocation of cell-penetrating peptides (CPPs) through plasma membranes of cells plays important roles. To reveal the mechanism of the translocation of a CPP, transporan 10 (TP10) through lipid bilayers, we examined the effects of mechanical properties of lipid bilayers on the entry of carboxyfluorescein (CF)-labeled TP10 (CF-TP10) into a giant unilamellar vesicle (GUV) (1). First, we examined the effect of tension in membranes on the entry of CF-TP10 into single GUVs. CF-TP10 entered the GUV lumen before membrane permeation of AF647, and the rate of entry of CF-TP10 increased with tension. The CF-TP10-induced fractional change in membrane area increased continuously with time until GUV rupture. A high mole fraction of cholesterol inhibited the entry of CF-TP10 into single GUVs by suppressing the translocation of CF-TP10 from the external to the internal monolayer, although higher concentrations of CF-TP10 induced formation of pores through which CF-TP10 rapidly translocated. We discussed the role of thermal fluctuations in membranes on the entry of CF-TP10 into single GUVs, and proposed a hypothesis on the mechanism that CF-TP10 translocates across a bilayer through transient hydrophilic prepores in the membrane (2).

(1) *Langmuir*, 33, 2433, 2017, (2) *PCCP*, 18, 13487, 2016

Posters

– 33. Membrane-active peptides –

P-1031

Self-association of histidine-modulated short arginine- and tryptophan-based antimicrobial peptidesL. János¹, M. Bacalum², F. Zorilă², A. Casian³, I. Turcu¹¹National Institute for Research & Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania; ²Horia-Hulubei National Institute of Physics and Nuclear Engineering, Bucharest, Romania; ³Supramolecular Organic and Organometallic Chemistry Center, Cluj-Napoca, Romania

Antimicrobial peptides (AMPs) are short sequences of aminoacids used as defense mechanism against foreign bodies. The selectivity of interaction with bacterial cells over mammalian cells underlines their significant potential in developing AMP-based drugs. Starting from a highly efficient tryptophan- and arginine-rich AMP (RRWWR-WWRR) seven new analogs were designed by substitution of tryptophans or arginines with histidines, and tested against three bacterial strains. Also, their cytotoxicity was evaluated and used to calculate therapeutic indexes. Molecular dynamics simulations showed that their antimicrobial activity can be correlated to the 3D-hydrophobic moment and to a structure-based packing parameter. The presence of histidine reduces the cytotoxic and hemolytic activity and enhances the aggregation of cationic AMPs around anionic lipids. Hence, the position of the histidine within the peptide sequence can be linked with AMP's mechanism of interaction with the membrane surface. We carried out preliminary in-silico experiments of AMP self-association at the membrane surface to identify potential mechanism of activity. Acknowledgements: NUCLEU Program, Project PN 16 30 02 03

P-1033

Elementary processes of antimicrobial peptide magainin 2-induced pore formation and its mechanismM. A. S. Karal¹, J. M. Alam², M. Hasan¹, V. Levadnyy^{1,3}, M. Yamazaki^{1,2}¹Grad. Sch. Sci. Tech., Shizuoka Univ., Japan; ²Res. Inst. Elec., Shizuoka Univ., Japan; ³Rus. Acad. Sci., Japan

* Magainin 2 (mag) forms pores in lipid bilayers, which is the main cause of its bactericidal activity. However, the mechanism of its pore formation is not well understood. Here we investigated the interaction of mag with single GUVs (1). The binding of mag to a GUV increased the fractional change in area of the GUV bilayer $D_{\text{el}}^{\text{ta}}$, which was proportional to mag surface concentration. This indicates that the rate constant of mag-induced pore formation k_p (2) greatly increased with $D_{\text{el}}^{\text{ta}}$. After the interaction of carboxyfluorescein (CF)-labeled mag (CF-mag) with a GUV containing AF647, the fluorescence intensity of the GUV rim due to CF-mag increased rapidly to a steady value, which remained constant for a long time, and just before the start of leakage of AF647 the rim intensity began to increase rapidly to another steady value. These results indicate that mag exists only in the outer monolayer until just before pore formation, which induces stretching of the inner monolayer. It is known that the stretching of lipid membrane can induce pore formation (3). Based on our model that the mag-induced stretching of the inner monolayer causes pore formation, we made a quantitative theory on k_p , which agrees with its experimental values. (1) *Langmuir*, 31, 3391, 2015, (2) *PCCP*, 16, 15752, 2014, (3) *PCCP*, 18, 13487, 2016

P-1032

Peptide features determining its ability of translocation and membrane pore formationI. Kabelka, R. Vácha

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Amphiphilic peptides can interact with phospholipid membrane and severely affect its barrier function by translocation or pore formation. This is particularly important for antimicrobial and cell-penetrating peptides as it can determine their lethality or ability to act as drug delivery systems against bacteria or pathological cells. However, the necessary peptide properties and conditions for membrane translocation and pore formation are not well understood. Using coarse-grained simulations, we have calculated the free energy of pore formation and translocation of amphiphilic helical peptides under various conditions. We found that the most effective in pore formation are peptides with length similar to membrane thickness. Moreover, the preferred peptide orientation in the pore and during the translocation was found to agree well with the hydrophobic mismatch rationalization. Long peptides were thus observed to orient parallel to membrane plane forming a 'double-belt' pore. The obtained understanding of peptide behavior at the membrane may be useful for the rational design of peptides that are more effective and specific against given target cells or bacteria.

P-1034

Translocation of cell penetrating peptides and calcium-induced membrane fusion share same mechanismA. Magarkar^{1,6}, C. Allolio^{1,2}, P. Jurkiewicz³, K. Baxova¹, R. Sachl³, D. Horinek², V. Heinz², R. Rachel², C. Ziegler^{2,4}, P. Jungwirth^{1,5}¹Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic; ²University of Regensburg, Regensburg, Germany; ³J. Heyrovsky Institute of Physical Chemistry, Prague, Czech Republic; ⁴Max-Planck Institute of Biophysics, Frankfurt, Germany; ⁵Tampere University of Technology, Tampere, Finland; ⁶University of Helsinki, Finland

Cell penetrating peptides have a unique potential for targeted drug delivery, therefore, mechanistic understanding of their membrane action has been sought since their discovery over 20 years ago.¹ While ATP-driven endocytosis is known to play a major role in their internalization,² there has been also ample evidence for the importance of passive translocation^{3–5} for which the direct mechanism, where the peptide is thought to directly pass through the membrane via a temporary pore, has been widely advocated.^{4,6} Here, we question this view and demonstrate that arginine-rich cell penetrating peptides instead enter vesicles by inducing multilamellarity and fusion, analogously to the action of calcium ions. The molecular picture of this penetration mode, which differs qualitatively from the previously proposed direct mechanism, is provided by molecular dynamics simulations. In addition, the kinetics of vesicle agglomeration and fusion by nonaarginine, nonalysine, and calcium ions are documented in real time by fluorescence techniques and the induction of multilamellar phases is revealed both via electron microscopy and fluorescence spectroscopy. We thus show that the newly identified passive cell penetration mechanism is analogous to vesicle fusion induced by calcium ions, demonstrating that the two processes are of a common mechanistic origin.

Posters

– 33. Membrane-active peptides –

P-1035

Structural properties of LL-37 derived AMPs define their antibacterial activity in blood plasmaN. Malanovic¹, R. A. Cordfunke², J. W. Drijfhout², A. de Breij², P. H. Nibbering², K. Zangger¹, K. Lohner¹¹University of Graz, Graz, Austria; ²Leiden University Medical Center, Leiden, Netherlands

Two LL-37 derived antimicrobial peptides, OP-145 and P148, show strong interaction with bacterial model membranes composed of the predominant Gram-positive membrane constituents: phosphatidylglycerol, lipoteichoic acid and peptidoglycan. Accordingly both peptides efficiently kill *Staphylococcus aureus* in phosphate buffer. However, in presence of blood plasma significant reduction of killing especially by OP-145 was observed. Furthermore, peptides exhibited different binding affinity to major component of human serum, albumin (HSA). In order to understand the different activity of the two peptides in blood plasma, essential for their application in humans, we aimed to correlate their antimicrobial activity with their structural properties. CD and NMR data indicated formation of α -helix for both peptides. Whereas P148 was always detected as stable aggregate, OP-145 aggregated only upon incubation either with model membranes or HSA at concentrations where it exerts its antimicrobial activity. In fact, stronger membrane permeability was observed by OP-145 when its aggregated form is stabilized by crosslinking with glutaraldehyde. Therefore, we can conclude that the antimicrobial activity for this group of peptides is strongly dependent on the stability of the aggregates.

P-1037

Entry of Lactoferricin B (4-9) into single vesicles and *E. coli* without damaging their membranesM. Moniruzzaman¹, M. Z. Islam¹, S. Sharmin¹, H. Dohra², M. Yamazaki^{1,3}¹Grad. Sch. Sci. Tech., Shizuoka University, Japan;²Res. Inst. Green Sci. Tech., Shizuoka University, Japan;³Res. Inst. Ele., Shizuoka University, Japan

Shorter versions of Lactoferricin B (LfcinB) such as LfcinB (4-9) have antimicrobial activity, but its mechanism is not well understood. To elucidate the mechanism of their antimicrobial activity, we investigated the interactions of LfcinB (4-9) with *E. coli* and giant unilamellar vesicles (GUVs). LfcinB (4-9) did not induce an influx of SYTOX green into cytoplasm of *E. coli*, indicating no damage in its plasma membrane. Next we observed the interaction of lissamine rhodamine B red-labeled LfcinB (4-9) (Rh-LfcinB (4-9)) with single cells of *E. coli* containing calcein using confocal microscopy. We found that Rh-LfcinB (4-9) entered the cytoplasm without leakage of calcein. We also investigated the interactions of Rh-LfcinB (4-9) with single DOPG/DOPC GUVs containing small vesicles and Alexa Fluor 647 (AF647). The results indicate that Rh-LfcinB (4-9) translocated through the GUV membrane and entered its lumen without leakage of AF647. Therefore, we can conclude that Rh-LfcinB (4-9) translocated across lipid membrane regions of the plasma membrane of *E. coli* to enter its cytoplasm without leakage and its antimicrobial activity is not due to damage of its plasma membrane. It highly contrasts with the mechanism of antimicrobial activity of LfcinB (Biochemistry, 54, 5802, 2015).

P-1036

Membrane permeation versus Amyloidogenicity: a multi-technique study of IAPPA. Martel¹, L. Antony², Y. Gerelli¹, L. Porcar¹, A. Fluitt², K. Hoffmann², I. Kiesel¹, M. Vivaudou³, G. Fragneto¹, J. J. de Pablo²¹The Institut Laue Langevin, 38042 Grenoble, France;²Institute for Molecular Engineering, University of Chicago, Illinois 60637, USA; ³Institut de Biologie Structurale, 38044 Grenoble, France

Islet amyloid polypeptide (IAPP) is responsible for cell depletion in the pancreatic islets of Langerhans, and for multiple pathological consequences encountered by patients suffering from type 2 Diabetes Mellitus. We have examined the amyloidogenicity and cytotoxic mechanisms of this peptide by investigating model-membrane permeation and structural effects of fragments of the human IAPP and several rat IAPP mutants. In vitro experiments and molecular dynamics simulations reveal distinct physical segregation, membrane permeation, and amyloid aggregation processes that are mediated by two separate regions of the peptide. These observations suggest a “detergent-like” mechanism, where lipids are extracted from the bilayer by the N-terminus of IAPP, and integrated into amyloid aggregates. The amyloidogenic aggregation would kinetically compete with the process of membrane permeation and, therefore, inhibit it. This hypothesis represents a new perspective on the mechanism underlying the membrane disruption by amyloid peptides, and could influence the development of new therapeutic strategies.

P-1038

Studying the role of negatively charged membranes on the mode of action of Esc 1b (1-18)

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Esculentin 1b is a peptide with 46 amino acids residues found in the skin of the hybrid frog *Pelophylax lessoane/ridibundus* but only the first 18 residues (GIFSKLAGKKLKNLLISG-NH₂) exhibit antimicrobial activity, without significant hemolysis. The mode of action of Esc 1b (1-18) was studied on membranes composed of different POPC:POPG molar ratios. Esc 1b (1-18) induced mainly permeabilization of GUVs composed of 1:1 POPC:POPG and POPG vesicles. Leakage of entrapped carboxyfluorescein and isothermal titration calorimetry indicated that the stoichiometry of the interaction and the membrane permeabilization extension were dependent on the POPG fraction. Electric measurements with planar lipid bilayers showed that the peptide induced ion passage only through 1:1 DOPC:POPG bilayers. The static and dynamic light scattering measurements showed accentuated peptide-induced aggregation of POPG LUVs, mild of 1:1 POPC:POPG and very low of POPC. The circular dichroism spectra showed a random coil conformation for peptide in water and in the presence of POPC LUVs, whereas the peptide acquires an α -helix conformation in the presence negatively charged vesicles. All results suggest that the mode of action of Esc 1b (1-18) is dependent on the negative charge concentration in the membrane surface. Financial support: FAPESP, CNPq and CAPES.

Posters

– 33. Membrane-active peptides –

P-1039

Model membranes and antimicrobial peptide interaction studied with a surface acoustic wave biosensor

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Biosensors based on surface acoustic waves (SAW) can substantially contribute to biosciences due to their high sensitivity for changes of mass, viscosity and electrical conductivity in near-surface layers [1].

With a biosensor employing surface guided bulk waves we studied the immobilisation of model membranes on different functionalizations of the sensor surface and their interaction with antimicrobial peptides in a fluid cell under flow conditions.

The antimicrobial peptide LL-32 derived from the protein hCAP18 of the human immune system has antimicrobial potency, but also immunomodulatory properties [2] whereas the fungal peptide toxin Candidalysin damages host cell membranes [3]. We investigated the underlying mechanisms utilizing reconstituted bacterial and eukaryotic membranes composed of different biological relevant lipids including lipopolysaccharides. Our results demonstrate the possibilities and limits of SAW biosensors and contribute to the understanding of the mode of action of LL-32 and Candidalysin in particular and membrane active peptides in general.

[1] Lange K et al. (2008) *Anal Bioanal Chem.* 391, 5

[2] da Silva FP, César Machado MC (2012) *Peptides* 36

[3] Moyes DL et al. (2016) *Nature* 532

P-1041

***In vitro* models of the Gram-negative outer membrane for antimicrobial research**N. Paracini¹, L. A. Clifton², S. W. Max², J. H. Lakey¹

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A realistic *in vitro* model of the Gram-negative bacterial outer membrane (OM) provides a versatile tool to study the properties of this complex biological barrier and understand its response to antibiotics. We used neutron reflectometry on a reconstituted asymmetric model of the OM to reveal the effects of the antimicrobial peptide Polymyxin B (PmB) on the structure of the bilayer. The antibiotic interacted with the model membrane in a temperature dependent manner, progressively disrupting the bilayer as it reached human physiological conditions. The transition of lipopolysaccharide (LPS) from a gel to a fluid state was shown to determine the extent of PmB interaction with the membrane *in vitro*, as confirmed by neutrons and infrared spectroscopy. The results obtained on the reconstituted membranes were compared with data collected on *E. coli* cells treated with PmB which confirmed a temperature dependent susceptibility to the antimicrobial. By correlating the effects of temperature on PmB bactericidal activity and its ability to disrupt the reconstituted OM *in vitro*, we further confirm the biological relevance of such models to understand membrane-active peptides mode of action.

P-1040

Recent developments in modification and characterisation of peptide-based artificial ion channelF. Otis¹, J.-D. Savoie¹, C. J. Moreau², M. Vivaudou², N. Voyer¹

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Channel proteins are essential for ion transport through cell membranes and are involved in numerous physiological functions. Compounds mimicking ion channel proteins have the potential to be used as therapeutics, antibacterials and signal transducing components in biosensors. Therefore, developing simple functional artificial ion channels using peptidic frameworks is attractive, because it allows easy molecular engineering and the modulation of channel properties such as ion selectivity and molecular recognition. Our group has developed an approach that uses 21-residue hydrophobic α -helical peptides as framework to orient macrocyclic neutral ligands (crown ethers) of different diameters that serve to create ion channels and to obtain ion selective transport across bilayer membranes.[1] We will present our efforts to characterise transport properties of the channels, and attempts to increase channel incorporation in cell membrane by adding small cell penetrating peptides at one extremity. The synthesis and other biophysical characterisation will also be presented.

[1] F. Otis, M. Auger, Normand Voyer, *Acc. Chem. Res.* **2013**, 46, 2934–294.

P-1042

Localization, organization, and permeabilization: membrane-specific activities of the AMP LL-32L. Paulowski¹, J. Wernecke^{2,1}, J. Bürck³, A. S. Ulrich³, B. A. Cornell⁴, T. Gutschmann¹

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Antimicrobial peptides (AMPs) comprise a peptide family with amazing skills and structural diversity. Their interaction with membranes covers a broad range of activities and is dependent on e.g. peptide concentration, buffer composition, membrane curvature, and lipid composition resulting in a multitude of models. LL-32, a truncated variant of the human cathelicidin, shows high membrane activity on model membranes of varying composition from unitary (DOPC) and binary (POPE/POPG) over ternary (DOPC/SM/Chol) to quaternary (DOPC/SM/Chol/PS) lipid systems. For membrane localization, asymmetric and immobilized GUVs were used along with oriented SRCD. The membrane organization was investigated by XRR-experiments on lipid multilayers. The susceptibility to peptide-induced membrane permeabilization was monitored by fluorescence quenching and dye release assays. It was possible to differentiate within conventional and new models: LL-32 interacts with DOPC membranes via the prominent carpet model whereas on cholesterol-rich membranes a novel mechanism fostered by lipid clustering is preferred. Lipid composition affects the membrane activity of α -helical AMPs reflecting the importance of lipid-specific models.

Posters

– 33. Membrane-active peptides –

P-1043

Nanoscale visualisation of peptoids interaction with multi-phase model lipid bilayers

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Peptoids are peptide-analogue molecules characterised by the fact that their side chain is connected to the nitrogen of the amide bond instead of the alpha-carbon. This feature makes them strongly resistant to proteolysis, and a promising class of antimicrobial agents. However, little is known about their conformation, including when interacting with molecules and membranes.

Here we use atomic force microscopy (AFM) to explore the interaction of peptoids with model DOPC/DPPC (1,2-dioleoyl-sn-glycero-3-phosphocholine/1,2-dipalmitoyl-sn-glycero-3-phosphocholine) mixed-phase membranes. The results provide molecular-level resolution insights into the spatial organisation of the different membrane domains. We find that the peptoid strongly interacts with the supported lipid bilayer and influences the spatial organization of the two phases, particularly affecting the gel-phase domains. This behavior appears concentration dependent, suggesting cooperation effects of the peptoid molecules. Moreover, exploiting the AFM tip-peptoid linking via thiol modification, it is possible to extract information about the peptoid conformation in the membrane.

P-1045

Investigating the mechanism of action of a novel antimicrobial peptide on live *E. coli* cellsA. L. B. Pyne^{1,2}, M.-P. Pfeil^{1,3}, I. Bennett², J. Ravi¹, B. Lamarre¹, B. W. Hoogenboom^{2,4}, M. G. Ryadnov¹¹National Physical Laboratory, UK; ²London Centre for Nanotechnology, University College London, UK;³Department of Biochemistry, University of Oxford, UK;⁴Department of Physics and Astronomy, University College London, UK

The rise of antimicrobial resistance (AMR) is a global issue requiring the development of new antimicrobial agents. Here the mechanism of a novel antimicrobial peptide, Tilamin, was investigated using atomic force microscopy (AFM). High resolution AFM was used to visualize the poration and exfoliation of the membrane of live *E. coli* cells in real time at nm resolution. Poration of the inner and outer membrane was observed on live cells, confirming results obtained in model membrane systems. These results suggest that transmembrane poration is not necessary for antimicrobial activity and reveal a distinct mechanism targeting the outer leaflet of phospholipid bilayers. This work offers a new mechanism for the design of novel antimicrobials which target one leaflet of the membrane.

[1] Alice Pyne, Marc-Philipp Pfeil, Isabel Bennett, Jascindra Ravi, Patrizia Iavicoli *et al*, *Chem. Sci.*, **8**, 1105–1115 (2017)

P-1044

Structural behavior of the peptaibol harzianin HK VI in a DMPC bilayer

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Microsecond molecular dynamics simulations of harzianin HK VI (HZ) interacting with a DMPC bilayer were performed at the condition of low peptide-to-lipid ratio. Two orientations of HZ molecule in the bilayer were found and characterized. In the orientation perpendicular to the bilayer surface, HZ assumes a rather 3_{10} -helical structure and induces a local thinning of the bilayer. When inserted into the bilayer parallel to its surface, HZ is located nearly completely within the hydrophobic region of the bilayer, and its structure is close to a β -bend ribbon. A combination of solid-state NMR and circular dichroism experiments indicated that the latter orientation is preferred. In agreement with that, an extended sampling simulation showed the parallel orientation to be a global minimum of free energy. The specific challenges of computer simulation of non-polar peptides like HZ are discussed briefly.

P-1046

Invertible micellar polymers interaction with membrane on microcavity supported lipid bilayer systemS. Ramadurai¹, A. Kohut², A. Voronov³, T. E. Keyes¹¹NCSR, Dublin City University, Dublin, Ireland; ²Dept. of Organic Chemistry, Lviv Polytechnic National University, Lviv, Ukraine; ³Coating and Polymer Materials, North Dakota State University, Fargo, ND, USA

Material transport such as drugs across the cellular membrane is highly regulated to protect the cell and drug carriers such as liposomes and polymeric micelles have been found to be effective drug delivery system. Amphiphilic invertible polymers (AIPs) can self-assemble into micellar structures at CMC and rapidly change structure in response to solvent polarity. Microcavity supported lipid bilayer (mSLB) have overcome the limitations of classical SLB and maintain the fluidity of membrane on both sides of the bilayer and thus providing better platform to study the interaction of AIPs with biological membrane using fluorescence lifetime correlation spectroscopy (FLCS) and electrochemical impedance spectroscopy (EIS). Here, we explored the interactions of 3 AIPs that exhibit different hydrophilic-lipophilic balance with membrane. The microcavity was made on PDMS for FLCS and on gold for EIS studies, and spanning lipid bilayer was spread over the aqueous filled cavity. We observe that both electrochemical impedance of the DOPC lipid membrane and mobility of the lipid are significantly modulated by the interaction of the polymers specifically PEG600-PTHF650 compare to D10 and S10, rationalise this behaviour on the basis of adsorption and penetration of the polymers through the bilayer.

Posters

– 33. Membrane-active peptides –

P-1047

Human lactoferricin derived peptides trigger apoptosis in malignant melanoma *in vitro* and *in vivo*
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Therapeutic options for hard-to-treat cancers are limited due to poor cancer toxicity and side effects caused by lack of specificity. Recently we demonstrated that the negatively charged lipid phosphatidylserine (PS) is specifically exposed by cancer cells and metastases, thus representing a novel target for cancer therapy – the basis for the design of cationic antitumor peptides derived from human Lactoferricin (hLFcin).

hLFcin derivatives show highly increased activity compared to the parent peptide hLFcin. We improved peptide specificity and efficacy towards cancer cells by variations of peptide length, net positive charge and hydrophobicity, consequently affecting secondary structure and killing mechanism. Our studies reveal characteristic features of hLFcin derivatives facilitating specific killing of cancer cells. Time dependent studies show that highly active but nonselective peptides act through a direct membranolytic mechanism whereas selective peptides trigger apoptosis, which is also membrane-mediated by primary interactions with PS and further intracellular targets. Furthermore cholesterol is crucial for the specificity of peptide-membrane interactions. Studies in mouse melanoma xenografts confirm the strong activity and selectivity of the designed peptides *in vivo*.

P-1049

Lung surfactant protein SP-C as a modulator of membrane structure: phase segregation and cholesterol
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Breathing is sustained by the presence of a lipid-protein complex lining the alveolar air-liquid interface. This material, termed lung surfactant (LS), establishes a complex meshwork of lipid assemblies that reduce surface tension, supporting respiratory dynamics with minimal energy requirements. Cholesterol represents a significant proportion of LS, but depending on its amount and conditions, it can impair LS interfacial activity, an effect reversed by the presence of SP-C, the smallest and most hydrophobic protein in LS. In this study, we made use of ²H nuclear magnetic resonance and electron spin resonance to analyse the effects of SP-C and cholesterol on lipid membranes mimicking LS composition. Our results demonstrated that the presence of SP-C led to phase segregation into a liquid crystalline phase and a highly ordered, possibly interdigitated, phase at 37°C. The ordered phase accommodated DPPC, POPC and POPG differently, an effect maximized by SP-C palmitoylation. In the presence of cholesterol, SP-C-containing membranes exhibited phase segregation at lower temperatures, which also seemed to selectively involve certain lipids. These results show how the combined action of SP-C and cholesterol modulates LS assemblies in terms of membrane structure and dynamics.

P-1048

A 3D model to test the therapeutic efficacy of adhirons in the treatment of HER2+ breast cancer
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University of Leeds, UK

Breast cancer (BC) is the most commonly diagnosed cancer amongst women in the UK. HER2 is overexpressed in 15-30 % of BC and is associated with poor prognosis. Trastuzumab dominates current treatment but has undesirable side effects, is expensive to make and requires mammalian cell culture for production.

Adhirons are a small, specific, thermostable and cost-effective alternative to monoclonal antibodies that do not require the use of animals or mammalian cell culture for their production. The scaffold has two variable loops that can be adapted to bind different targets. Eight Adhirons that bind HER2 were identified and their potential for the treatment of HER2+ BC is being investigated.

To test therapeutic efficacy a 3D model of BC will be used. 3D culture can recapitulate tumour heterogeneity more accurately than 2D culture. As proof of principle 3D spheroids were generated from the BT-474 HER2+ breast cancer cell line using epiFL, a fully humanised culture medium. Our long-term goal is to create a reproducible *in vitro* model of BC using a 3D scaffold combined with microfluidics that will allow high throughput screening of Adhirons against HER2, replacing the use of animals in early phase drug testing.

P-1050

Solid-state NMR study of live bacteria in the presence of antimicrobial peptides
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Antimicrobial peptides (AMPs) have been extensively studied as alternatives to traditional antibiotics. Solid-state NMR is used to characterise their effect on lipid bilayers, which are the primary target, but correlation with *in vivo* situations is tentative in view of the complex effects of changes in sample conditions (such as pH, temperature, lipid composition or peptide concentration). We have used ³¹P solid-state NMR to study the impact of the AMP maculatin 1.1 on live *E. coli* and *S. aureus* bacteria. A combination of dynamic filtering and paramagnetic reagents was used to identify signals from molecular species such as nucleic acids, lipids or inorganic phosphate. At peptide concentrations below the minimum inhibition concentration (MIC), a significant impact on the DNA packing of *E. coli* and *S. aureus* was observed. Interestingly, the peptide effect on *S. aureus* was significantly less, particularly on the phospholipid signals, despite having a lower MIC than *E. coli*. Also, peptide treatment in *E. coli* induced the production of a new phosphorus signal, consistent with a phosphonate species. Overall, an extensive solid-state ³¹P NMR study of live bacteria and preliminary in-cell DNP-NMR of labelled peptides have provided new insights into the bactericidal mechanisms of AMPs.

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– 33. Membrane-active peptides –

P-1051

Cell-density dependence of host-defense peptide activity and selectivityF. Savini¹, V. Luca², A. Bocedi¹, R. Massoud³, Y. Park⁴, M. L. Mangoni², L. Stella¹¹University of Rome Tor Vergata, Department of Chemical Sciences and Technologies, 00133 Rome, Italy; ²Sapienza Rome University, Department of Biochemical Sciences "A. Rossi Fanelli", 00185 Rome, Italy; ³Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, 00133 Rome, Italy; ⁴Chosun University, Department of Biotechnology, 501-759 Gwangju, South Korea

Host-defense peptides (HDPs) are promising compounds against multidrug-resistant microbes. In vitro, their bactericidal and toxic concentrations are significantly different, but this might be due to the use of separate assays, with different cell densities. For experiments with a single cell type, the cell-density dependence of the active concentration of the DNS-PMAP23 HDP could be predicted based on the water/cell-membrane partition equilibrium and exhibited a lower bound at low cell counts. On the basis of these data, in the simultaneous presence of both bacteria and an excess of human cells, one would expect no significant toxicity, but also inhibition of the bactericidal activity due to peptide sequestration by host cells. However, this inhibition did not take place in assays with mixed cell populations, showing that for the HDP esculentin-1a(1–21)NH₂, a range of bactericidal, nontoxic concentrations exists and confirming the effective selectivity of HDPs. Mixed-cell assays might be necessary to effectively assess HDP selectivity.

P-1053

Cholesterol-conjugated peptide inhibitors of influenza virus: biophysical characterizationP. M. Silva¹, M. C. Marques¹, M. T. Augusto¹, M. Porotto², A. Moscona³, N. C. Santos¹¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal; ²Department of Pediatrics, Columbia University Medical Center, New York, USA; ³Departments of Pediatrics, Microbiology & Immunology, and Physiology & Cellular Biophysics, Columbia University Medical Center, New York, USA

The influenza virus is responsible for respiratory diseases affecting millions of people worldwide. Infections can be controlled by vaccines and antiviral drugs. However, the virus is constantly under mutations, leading to growing resistance to antivirals. Influenza hemagglutinin (HA), a potential target for antivirals, is involved in receptor binding and promotes the pH-dependent fusion of virus and cell membranes after endocytosis. Cholesterol-conjugated HA-derived membrane fusion inhibitor peptides have been previously studied on live viruses. Three HA-derived peptides were analyzed by fluorescence spectroscopy, including membrane partition to assess the interaction with biomembranes systems, human blood cell-binding (followed with a dipole potential probe) and preferential localization in lipid bilayers (using aqueous-soluble and lipophilic quenchers). The conjugated peptides were more active than the unconjugated. (Influenza-PEG4)2-Chol peptide presented higher membrane affinity at pH 7.4. However, that affinity decreased in acidic environment, a possible advantage due to membrane release after viral endocytosis. Our results provide new insight into possible strategies toward the development of new influenza virus inhibitors.

P-1052

Infrared reflection absorption spectroscopy reveals structural transitions in membrane proteins

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Infrared Reflection Absorption Spectroscopy (IRRAS) is a versatile technique to study the organization of interfacial films on a molecular or even sub-molecular level. It provides important information to understand macroscopic properties of interfacial layers. IRRAS combines the film balance technique with IR spectroscopy, which provides structural information, reveals the presence of molecules, their interactions, their conformation and their phase state. In addition, and in contrast to solution IR spectroscopy the orientation of molecules or molecular moieties can be determined by IRRAS. This is due to the perfectly flat geometry of the model system and the possibility to control polarization and incidence angle of the IR light.

This technique was used to determine conformation and orientation in which fusion peptides bind to lipid membranes [1]. Furthermore, I will demonstrate how the orientations of membrane binding proteins can be determined and even structural rearrangements within large proteins during the binding process can be investigated in nearly natural conditions, i.e. physiological temperature, desired buffer composition and low protein concentration [2].

[1] M. Rabe, *Langmuir*, **2014**, 30, 7727[2] M. Hoernke, *PNAS*, **2017**, in press

P-1054

Rubber particle proteins, HbREF and HbSRPP, interact differently with lipids extracted from RRIM600K. Wadeesirisak¹, S. Castano², K. Berthelot², S. Liengprayoon³, C. Bottier⁴, S. Lecomte²¹Faculty of Agro-Industry, Kasetsart University, 10900 Bangkok, France; ²CBMN, allée Geffroy St Hilaire, 33600 Pessac, Thailand; ³KAPI, Kasetsart University, 10900 Bangkok, Thailand; ⁴CIRAD UMR IATE- HRPP -Kasetsart University, 10900 Bangkok, Thailand

Latex tapped from *Hevea brasiliensis* (rubber tree) contains rubber particles dispersed in C-serum. Two major proteins are abundant at the surface of *Hevea* rubber particles namely, the HbREF and the HbSRPP, which are mainly located at the surface of large rubber particles and small rubber particles, respectively. Lipids extracted from rubber particles include phospholipids (PL), glycolipids (GL) and neutral lipids (NL) [5].

We used Brewster angle microscopy and PM-IRRAS to investigate the interactions between recombinant REF and SRPP with native lipids extracted and purified from *Hevea* RRIM600 latex collected in the Chanthaburi province, Thailand. Monolayers of native phospholipids, glycolipids and neutral lipids were successfully formed at the air-water interface. SRPP interacts mostly in surface with all types of lipids (PL, GL or NL), without modification of its secondary structure. In contrast REF inserts deeply in the lipid monolayer with all lipid classes. When it interacts with NL, REF is able to switch from a conformation in α -helices to β -sheet structures.

Posters**– 33. Membrane-active peptides –****P-1055****Membrane activity of the fungal peptide toxin Candidalysin**J. Wernecke^{1,2}, L. Paulowski¹, C. Nehls¹, J. R. Naglik³, B. Hube⁴, T. Gutschmann¹¹Deutsches Elektronen-Synchrotron, Germany; ²Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Germany; ³Dental Institute, King's College London, UK; ⁴Department of Microbial Pathogenicity Mechanisms, Hans Knöll Institute, Germany

For decades, researchers have been trying to elucidate critical determinants of the pathogenicity of human fungi. Only recently, has an international consortium of scientists succeeded in identifying such a factor – a peptide toxin secreted by the clinically important fungus *Candida albicans*. This peptide Ece1-III plays a crucial role during fungal infections of human mucosae. The peptide's virulence manifests in the direct damage of epithelial membranes, in the stimulation of a danger response signalling pathway and in the activation of epithelial immunity. We provided the first insights into the direct interaction between Ece1-III and lipid membranes [1]. The peptide's amphiphilic alpha-helical structure is described as a prerequisite for its binding to lipid membranes. In consequence of the initialised binding, the peptide inserts between the lipid head groups and aligns parallel to the bilayer surface. Upon increasing surface accumulation, however, the helix starts to penetrate the bilayer with oblique inclination. As a result, the bilayer is destabilised and transient local collapses occur. This carpet-like disintegration eventually leads to the disruption of the entire membrane.

[1] Moyes et al., Nature 532, 64–68 (2016).

P-1056**Three conserved residues of influenza fusion peptide alter its behavior at the membrane interface**R. Worch¹, A. Filipek¹, J. Krupa¹, A. Szymaniec¹, P. Setny²¹Institute of Physics, PAS, Warsaw, Poland; ²Centre for New Technologies, University of Warsaw, Poland

To release the genetic material, a process of fusion between influenza envelope and host cell membrane occurs, which is mediated by hemagglutinin (HA) protein. The N-terminal fragment of HA2 subunit, directly interacting with the membrane, is named a fusion peptide (HAfp). Its C-terminal part contains three residues (W21-Y22-G23), which are conserved among various serotypes. Peptide length has an influence on its structure: HAfp1-20 forms a boomerang in contrast to a tight helical hairpin formed by HAfp1-23. We studied the effect of peptide length on fusion properties, its structural dynamics, and partitioning to the bilayer. By means of MD simulations and spectroscopic measurements, we showed that three C-terminal residues in HAfp1-23 promote the formation of hairpin structure. Using a novel fusion visualization assay based on FLIM microscopy on giant unilamellar vesicles (GUV), we observed that HAfp1-23 promotes fusion to a higher extent than HAfp1-20. Moreover, we report cholesterol-enriched domain formation exclusively by the longer fusion peptide. This redistribution of membrane components in fluid phases is likely to play a role during membrane fusion.

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– 34. Why disorder matters –

P-1057 (O-202)

Mapping the link between disorder and function of an IDP-network with single-molecule spectroscopy

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Intrinsically disordered proteins (IDPs) are ubiquitously found in eukaryotic systems. Their lack of a well-defined structure suggests that their broad conformational ensemble is functionally advantageous. A particularly important system is the IDP-network formed by the basic helix-loop-helix leucine zipper (bHLH-LZ) domains of c-Myc, Max, and Mad, which are major regulators of transcriptome dynamics. Here, we use single-molecule fluorescence resonance energy transfer to investigate the link between the polymer properties of the bHLH-LZ domains of c-Myc, Max, and Mad and the process of coupled binding and folding that leads to functional complexes. In contrast to archetypal IDPs, all three proteins form densely collapsed ensembles under physiological conditions that are dominated by strong attractive electrostatic interactions as quantified using polymer theory. Importantly, the ionic strength sensitivity of the disordered ensembles has pronounced consequences for their functional interactions within the network since salt modulates the binding affinity by almost three orders of magnitude. Our results suggest that the properties of the disordered ensemble and the stability of the functional complexes are strongly correlated.

P-1059 (O-204)

Structural and dynamic aspects of antibody recognition of intrinsically disordered antigens

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Intrinsically disordered proteins are abundant in *Plasmodium* and related pathogens^[1] and are bona fide vaccine candidates.^[2] Merozoite surface protein 2 (MSP2) is an intrinsically disordered membrane antigen of *P. falciparum* ^[3] that elicits a protective response in humans. Crystallography and NMR show^[4] that recognition of a conserved N-terminal epitope by mAb 6D8 is incompatible with its membrane-bound conformation,^[5] suggesting a mechanism by which parasite MSP2 escapes 6D8 recognition. NMR also identifies transient, strain-specific interactions between the 6D8 mAb and more remote regions of MSP2. The conserved C-terminal region of MSP2 is recognised by mAbs 4D11 and 9H4. 4D11 binds to merozoites much more strongly than 9H4. A crystal structure of 4D11 Fv bound to the epitope NKENCGAA reveals the possible conformation of the C-terminal region of MSP2 on the parasite,^[6] underpinning optimization of MSP2 as a vaccine candidate.

[1] Feng et al *Mol. Biochem. Parasitol.* **2006**, *150*, 256 [2] MacRaild et al *Structure* **2016**, *24*, 148 [3] Zhang et al *J. Mol. Biol.* **2008**, *379*, 105 [4] Morales et al *Sci Rep* **2015**, *5*, 10103 [5] MacRaild et al *Biochim. Biophys. Acta* **2012**, *1818*, 2572 [6] Seow et al *J. Mol. Biol.* **2017**, *429*, 836.

P-1058 (O-203)

Interplay Between Surface Solvation and Molecular Recognition in IDPs

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Solvation dynamics tunes bio-molecular dynamics. Such dynamics play a pronounced role for Intrinsically Disordered Proteins (IDPs) as their molecular-recognition goes beyond classical ‘structure-function’ paradigm. We interrogate the interplay between solvation dynamics and molecular-recognition in IDPs; using a combination of site-selective ultrafast fluorescence and all-atom MD simulations. We probed surface water dynamics in IDPs and its attenuation upon partner binding for two IDPs involved in nucleocytoplasmic transport, Nup153FG (N153) and IBB which have as a common binding partner the nuclear transport receptor (NTR) Importin β (I β), despite having different binding modes. N153 binds I β through a set of ultrafast transient multivalent interactions retaining its disorder while IBB forms a helix upon binding I β . Solvent dynamics in N153-I β complex were unperturbed relative to the unbound state while in IBB-I β complex relative slowdown of solvation was seen. This shows a direct correlation between interfacial water dynamics and plasticity of the complexes.

P-1060

Denatured protein dynamics investigated with neutron spin-echo spectroscopy NSE

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The process of protein folding is highly dependent on the amino acid composition as well as on the solution condition, especially on the presence of denaturant. Our approach is to describe the dynamics and structure of the unfolded protein using polymer theory. Neutron scattering experiments have been performed as a function of denaturant type and denaturant concentration which has proved to be promising as demonstrated by single-molecule Förster resonance energy transfer FRET experiments¹. Small-angle scattering and NSE results of native and denatured Bovine Serum Albumin BSA in solution and at various concentrations of guanidine hydrochloride GndHCl will be presented. NSE studies of denatured BSA reveal a significant contribution of internal dynamics to the overall global diffusion that is clearly missing in the native state. We successfully showed that models from polymer theory are suitable for the interpretation of the observed motions. While BSA at 6M GndHCl follows quasi pure Zimm dynamics, dynamics at 4M GndHCl are best interpreted with a Zimm model including internal friction that reveals that an offset is required as contribution to all relaxation times.

Reference:

[1] A. Soranno et al., PNAS. *109*, 44, (2012)

Posters

– 34. Why disorder matters –

P-1061

How structural order/disorder transitions modulate interactions in RSV phosphoproteinC. Cardone¹, N. Pereira¹, S. Lassoued¹, C.-A. Richard², J. Fix², F. Bontems¹, M. Galloux², J.-F. Eleouet², C. Sizun¹¹Institut de Chimie des Substances Naturelles, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France; ²Unité de Virologie et Immunologie Moléculaires U892, INRA, Jouy-en-Josas, France

Human Respiratory Syncytial Virus (RSV), of the order *Mononegavirales*, is the main viral cause of lower respiratory tract illness worldwide. To design specific treatment by antiviral drugs, we have undertaken to characterize the viral RNA polymerase complex (Ouzougoun-Oubari et al, 2015). Here we report a structural and dynamic description of RSV phosphoprotein (P), the main RSV polymerase co-factor, by NMR. We show that intrinsically disordered N- and C-terminal domains amount to 80 % of P, outside of the oligomerization domain. These display striking dynamic heterogeneity. All transient or stable helices mediate transient internal long-range contacts, possibly achieving compaction of P in the absence of a defined tertiary structure. Importantly even very transient secondary structure elements in P provide protein binding sites that are specifically recognized by viral proteins belonging to the polymerase complex. However, binding does not necessarily induce structuration. Recognition of the RSV nucleocapsid by P proceeds via its C-terminus, an extended region of P without any secondary structure propensity. In this case, multiple unspecific contacts contribute to the affinity, which is expected to be low to facilitate polymerase processivity (Pereira et al, 2017).

P-1063

Conformational biases of tau protein's microtubule binding repeat regionsO. Cehlar^{1,2}, R. Skrabana^{1,2}, M. Novak^{1,2}¹Axon Neuroscience R&D Services, Dvorakovo Nabrezie 10, 811 02 Bratislava, Slovakia; ²Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia

Accumulation of intrinsically disordered protein tau in the form of insoluble aggregates is a common feature of neurodegenerative tauopathies. Monoclonal antibody DC8E8 is able to inhibit tau-tau interaction and therefore it holds promise for the immunotherapy of Alzheimer's disease. The active vaccine based on the DC8E8 epitope peptide has successfully passed the phase 1 clinical trial. Minimal epitope of DC8E8 represents amino acid motif HXPGGG that is present in each of the four microtubule binding repeats (MTBRs) of tau. However, the affinity of DC8E8 for its MTBR epitopes differs and descends as follows: MTBR2 > MTBR1 > MTBR3 > MTBR4. These differences in the antibody affinity for highly homologous epitopes can be attributed to different conformational biases of epitope peptides for the bound conformation. We have crystallized the complexes of MTBR peptides with DC8E8 Fab fragment to elucidate its binding mode. We have further performed molecular dynamics simulations of all four MTBR peptides and compared the percentage of sampled bound-like conformation with the antibody affinity. Unravelling the unique mode of recognition of DC8E8 antibody and conformational biases of tau protein repeat regions can aid to reveal the hindered structural features of tau protein biology.

P-1062

Linking the protein free energy landscape to the intracellular milieuD. A. Catici¹, J. Mason¹, V. L. Arcus², C. R. Pudney¹¹Department of Biology and Biochemistry, Faculty of Science, University of Bath, Bath, BA2 7AY, UK; ²School of Science, Faculty of Science and Engineering, University of Waikato, Hamilton 3240, New Zealand

Understating the protein specific thermodynamic profile constitutes a key step towards answering fundamental questions about the structure, function and dynamics of biological molecules inside living cells. It has become apparent that the physical factors governing the complex intracellular environment (eg. macromolecular crowding, changes in pH, presence of osmolytes) all contribute to folding and ensuring proper functioning of proteins. Here we report the effects of the intracellular milieu on the functional conformational change of NF- κ B essential modulator (NEMO). Being the main activator of the NF- κ B signalling pathways, NEMO is responsible for regulation of the immune and nervous system. We examine the effect of crowding, viscosity and presence of osmolyte on a number of thermodynamic parameters to gain insight into the intricate relationship between proteins and their environments. In the case of NEMO, we describe the intracellular milieu to act as an additional control in modulating protein structure and function. We show how mimics of the intracellular milieu significantly alter thermodynamics of NEMO functional conformational change, highlighting the importance of considering a variety of cellular parameters when reporting on protein structure and dynamics.

P-1064

Intrinsically disordered signaling proteins can exhibit emergent cooperativity, sequential bindingL. Clemens¹, O. Dushek², J. Allard¹¹University of California - Irvine, USA; ²University of Oxford, UK

Many signaling proteins have intrinsically disordered regions. Despite lack of structure, some of these exhibit complex non-linear behavior, including cooperativity and sequential binding. For example, T Cell Receptor zeta chain has six tyrosines and exhibits an approximately 100-fold enhancement from first to sixth phosphorylations. Here, we model the zeta chain with a simplified theta-solvent freely-jointed chain model coupled to idealized spherical binding enzymes. We explore three experimentally-motivated models: (1) phosphorylation stiffens nearby bonds, (2) phosphorylation modulates electrostatic interaction with the membrane, and (3) phosphorylation allows enzymes, e.g. Zap-70, to remain bound to the tyrosines. We find that entropic flexibility alone can lead to cooperativity, anti-cooperativity and sequential binding. In all cases, the presence of a nearby membrane further enhances these effects. Some of this enhancement is due to the specific locations of tyrosines along the chain. This introduces the possibility that receptor function could be tuned by changing the distance between tyrosines in the disordered sequence. Our results demonstrate the disordered regions themselves may act as modules in signal transduction cascades.

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– 34. Why disorder matters –

P-1065

Structure and Self-Assembly of Elastin-Like Peptides: A Joint Molecular Dynamics and NMR StudyQ. Huynh^{1,2}, S. Reichheld¹, S. Rauscher^{2,3}, S. Sharpe^{1,2}, R. Pomès^{1,2}¹Hospital for Sick Children, Canada; ²University of Toronto, Canada; ³Max Planck Institute for Biophysical Chemistry, Germany

Elastin endows skin, arteries, the lung, and the uterus with extensibility and elasticity. Elastin and elastin-like peptides are structurally disordered and self-aggregate via liquid phase separation. Despite extensive study, the structural basis for the self-assembly and the mechanical properties of elastin remains unclear. As an essential step towards elucidating the structural ensemble of elastin, we combine molecular dynamics simulations and NMR spectroscopy to study an elastin-like peptide modelled after the sequence of alternating hydrophobic and cross-linking domains of elastin. Computational and spectroscopic results are in excellent agreement. Although the peptide is highly disordered, it possesses a significant propensity for local secondary structure. The cross-linking domains are characterized by fluctuating helical structure, whereas the hydrophobic domains form sparse and transient hydrogen-bonded turns. As a result, the individual domains are collapsed but not compact, and they remain disordered and hydrated despite their predominantly hydrophobic character. These findings resolve long-standing controversies regarding the structure and function of elastin and afford insight into the physical and structural basis for the phase separation of disordered proteins.

P-1067

Secondary structure prediction of disordered proteins by CD spectroscopyA. Micsonai¹, N. Murvai², É. Bulyáki¹, B. Szabó², F. Wien³, M. Réfrégiers³, Y.-H. Lee⁴, Y. Goto⁴, K.-H. Han⁵, P. Tompa², Á. Tantos², J. Kardos¹¹MTA-ELTE NAP B Neuroimmunol. Res. G., Dept. Biochem., Eötvös Loránd Univ., Hungary; ²Inst. Enzymol., HAS, Hungary; ³Synchrotron SOLEIL, France; ⁴Inst. Prot. Res., Osaka Univ., Japan; ⁵KRIBB, South Korea

Intrinsically unstructured proteins (IUPs) play important roles in the living cell. CD spectroscopy is an inexpensive and fast technique for the study of the secondary structure of proteins. It is especially useful when the use of high-resolution techniques, such as X-ray crystallography or NMR spectroscopy are limited, such as the case of IUPs. However, the CD spectrum analyzing algorithms usually overestimate the β -structure content, making the study of disordered proteins problematic. Recently, we developed a new method called BeStSel for the secondary structure estimation and fold recognition from the CD spectra (Micsonai et al., PNAS, (2015) 112, E3095), which provides superior results for any of the secondary structure elements. Here, we have developed further this algorithm to improve its performance on IUPs. We have recorded the CD spectra of disordered proteins and modelled their structure by MD simulations. BeStSel algorithm was re-optimized on the extended reference database. Statistical evaluation proved that the algorithm is able to distinguish the otherwise spectrally similar disordered structure from the highly twisted β -sheets.

P-1066

Why would nature give two PDZ domains to the “Golgi reassembly and stacking protein”?L. F. S. Mendes^{1,2}, C. Redfield², P. J. Judge², A. Watts², A. J. Costa-Filho¹¹University of Sao Paulo, Brazil; ²University of Oxford, UK

The Golgi complex is an organelle responsible for receive synthesized cargo from the endoplasmic reticulum for subsequent modifications, sorting and secretion. A family of proteins named Golgi Reassembly and Stacking Proteins (GRASP) is essential for the correct assembly and laterally tethering of the Golgi cisternae, a necessary structuration to keep this organelle working correctly. The GRASP structure is mainly composed of two regions, with the N-terminal region being formed by two PDZ domains connected by a short loop. Even though there are now a few crystal structures solved for the N-terminal domain, it is surprising to notice that very limited information is available regarding the structural differences between the two PDZs in the N-terminal, which is the main functional region of this protein. We used solution NMR and synchrotron radiation circular dichroism to probe the structural differences between both PDZs, but now in solution. We clearly observed that both PDZs behave far from been equal, with the first PDZ being significantly more dynamic and unstable compared with PDZ2. We have also shown that there are significant more disordered regions in PDZ1, what gives a new insight related to the PDZ1 promiscuity of interacting with much more protein partners than PDZ2.

P-1068

Investigation of hemorehological changes in chronic obstructive pulmonary diseaseD. K. Saribal¹, N. Bahtiyar¹, M. C. Akyolcu¹
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Chronic obstructive pulmonary disease (COPD), is characterized by reduced maximal expiratory flow and slow forced emptying of the lungs, leading to further morbidity and significant mortality. Hypercoagulability disorders can be occurred in COPD. Erythrocyte count, hemoglobin, hematocrit, plasma and blood viscosities are varied markers in hypercoagulability. In our, it has been investigated that erythrocyte count, hemoglobin level, hematocrit, plasma and blood viscosity were investigated as state of hypercoagulability in COPD.

25 COPD patients and 25 healthy subjects were included in the study. Plasma viscosity was measured in a Harkness viscometer. Blood viscosity was measured the shear-stress at different shear-rates with a Brookfield viscometer. Erythrocyte count, hemoglobin, hematocrit were counted with an automatic counter.

Erythrocyte count, hemoglobin, hematocrit were found higher in COPD group comparing to control group, but these values are not statistically significant. In addition, blood and plasma viscosity results did not significantly differ between patient and control groups.

It has been widely demonstrated that hyperviscosity and hypercoagulability occur in the blood of patients with COPD, but our opinion this disorders may happened in patients with advanced COPD.

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– 34. Why disorder matters –

P-1069

Flexible recognition of a flexible target: inhibition of tau protein oligomerization by DC8E8

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Tau protein oligomeric forms are likely the toxic agents underlying neurodegeneration in Alzheimer's disease, therefore, an immunotherapy targeting pathogenic tau epitopes holds promise for a disease modifying treatment. Strong evidences exist that tau oligomerization is nucleated via hexapeptides VQIINK and VQIVYK in the microtubule-binding tau region. DC8E8 is a mouse anti-tau monoclonal antibody effectively blocking tau-tau interaction in vitro. DC8E8 recognizes four homologous epitopes HxPGGG, two of them in the immediate vicinity of the aggregation-promoting hexapeptides. Tau active vaccine AADvac1 derived from an epitope component of DC8E8 is under clinical development. We have determined several X-ray structures of DC8E8 Fab and its complexes with tau peptides and deduced steps of complex formation. Partially flexible antibody loop CDRH3 participates on specific tau recognition. Tau protein chain may adopt the type I beta-turn by residues GGGS. The bulky CDRL1 loop of the antibody remains conformationally unstable in the complex and forms an entropic shield of the beta-structure forming hexapeptides adjoining the epitope. The results allowed a mechanistic insight into DC8E8 anti-aggregation activity and showed a strategy used by immune system to recognize a flexible target.

P-1071

Focus on androgen receptor N-terminal flexible domain: implication for neurodegeneration

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The androgen receptor is a transcription factor implicated in the etiopathogenesis of prostate cancer, androgen insensitivity syndrome and spinal bulbar muscular atrophy. The latter is a rare neurodegenerative disease affecting male adults with a rate of 1 in 40000. The genetic hallmark of the disease is the expansion of the CAG triplet in exon 1 of the androgen receptor gene, yielding a protein with an extended polyglutamine tract (polyQ) in the disordered N-terminal region of the protein. So far, the causes of the disease have not been identified, yet transcriptional dysregulation is known to play a role. The aim of this project is to analyse the molecular details favouring protein aggregation in disease. In detail, we are investigating the role of phosphorylation of the N-terminal domain of the androgen receptor, focusing on the structural and functional consequences of phosphorylation on the biology of the protein, in the presence of native and expanded poly-glutamine stretches. Our data show that phosphorylation at Serine 96 by Cdk2/CyclinE increases protein stability in motor-neuron derived cells MN-1 stably expressing human androgen receptor, and that this event is toxic in the presence of expanded polyQ tract.

P-1070

Structural disorder of monomeric α -synuclein persists in mammalian cells

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The native structure of Parkinson's disease protein α -synuclein is a matter of debate. After delivering ¹⁵N-labelled α -synuclein in mammalian cells, we characterized its chemical modifications, its conformational behavior and its interactions with the cellular environment using both in-cell NMR and EPR [1,2]. We have observed that α -synuclein adopts the same conformations and dynamics in cells than *in vitro*, and protects its central aggregating region from cellular interactions. However, oxidative conditions do have irreversible impacts on α -synuclein post-translational modifications, which we monitored at the atomic scale using our recent protocols [3,4]. We will discuss it with regard to recent structural studies in specific organelles environments.

References:

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2. Binolfi A et al. *Nat Commun*, 7, 10251 (2016)
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P-1072

SNPs in 5-HTR and GRIN2B associated with risk of cognition dysfunction in electric workers

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The CNS is a potential target of interaction between biological systems and ELF-MF. Some studies found that there were differences in the change of brain function between individuals after electromagnetic radiation. In the study, some abnormal characterizations of ECG in operating and repairing groups were observed compared to control group, which was shown as arrhythmia, bradycardia, T wave abnormalities, etc. The N2 latency of P300 was prolonged and the contents of LENK were increased in repairing group compared to control and operating groups, the contents of BNP in operating group were increased compared to control group. The latency of N2 and P3 in TT genotype of rs6313, the N2 latency and the contents of LENK in GG genotype of rs6295, the N2 latency in CC genotype of ENS10557853 were prolonged, with the increase of exposure dosage respectively compared to CC, CG and TT genotypes. Above all, 5-HT_{1A}R in rs6295 G allele, GRIN2B in ENS10557853 C allele and 5-HT_{2A}R in rs6313 T allele might conduce to the cognition retardation in the electric workers.

Key Words: ELF-EMF; 5-HTR; GRIN2B; polymorphism; cognition

(This work was supported by National Natural Science Foundation of China 61571455)

Posters– 34. *Why disorder matters* –**P-1073****Is there relation between heavy metals and lipid peroxidation in laryngeal cancer?**A. K. Yoldas¹, S. Toplan¹, O. Aslan², B. Aydemir³,
D. K. Saribal¹¹Istanbul University Cerrahpasa Medical Faculty, Department of Biophysics, Turkey; ²Istanbul University Medical Faculty, Turkey; ³Sakarya University Medical Faculty, Department of Biophysics, Turkey

Background: There are signs related to relation between lipid peroxidation and heavy metals in cancer. To screen the relation between heavy metals such as zinc(Zn), copper(Cu), lead(Pb), cadmium(Cd) levels and malondialdehyde(MDA) concentration as lipid peroxidation marker in laryngeal cancer.

Method: In present study, considered element measurements and malondialdehyde concentration determinations were realized in blood samples of larynx cancer patients and healthy controls.

Findings: Where Cu concentration found to be higher in cancer group than controls but Zn levels were determined at lower level in serum samples. Lead and cadmium levels in cancer group was statistically higher compare to control values in blood samples. On the other hand malondialdehyde concentrations was found higher both in plasma and blood samples of cancer group.

Results: Our results show that higher MDA levels may lead to development of malign tumors. Possible reason of such a situation could explained be with the inhibitory effect of heavy metals on antioxidant defense of body.

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Posters

– 35. Physics of cancer –

P-1074 (O-208)

The application of scanning near field infrared microscopy to cancer

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There is an international need to improve the diagnosis of cancer. Traditional methods rely on the examination of excised tissue by histologists, two of whom, for some cancers, typically agree on a diagnosis only 70% of the time. False positives can lead to serious but unnecessary surgery and false negatives can be fatal. Fortunately it is becoming clear that the diagnosis of cancer can be improved by analysis techniques based on the examination of tissue using infrared (IR) imaging technology. Recently it has been shown that two methods of IR imaging, scanning near field optical microscopy (SNOM) using an IR free electron laser [1] and Fourier transform IR (FTIR), are able to discriminate between normal, dyskaryotic and cancerous cervical tissue [2]. The two IR imaging approaches have different strengths and weaknesses. FTIR yields information from several thousand wavelengths but at poorer spatial resolution whereas SNOM provides excellent spatial resolution but over a smaller number of wavelengths. The two techniques will be compared in studies of a single cancer cell.

1 A.D. Smith, et. al. App. Phys. Lett. **102** 053701 (2013)2 D.E. Halliwell, et.al. Nature Scientific Reports, **6** 29494 (2016)

P-1076 (O-210)

AFM and graph analysis to study P-cadherin/SFK mechanotransduction signalling in breast cancer cellsF. A. Carvalho¹, A. S. Ribeiro², J. Figueiredo², R. Carvalho², T. Mestre³, J. Monteiro², A. F. Guedes¹, M. Fonseca³, J. Sanches³, R. Seruca², J. Paredes², N. C. Santos¹

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Forces mediated by cell-cell adhesion molecules, as cadherins, play a crucial role in preserving normal tissue architecture. In breast cancer, P-cadherin overexpression increases *in vivo* tumorigenic ability, as well as *in vitro* cell invasion, by activating Src family kinase (SFK) signalling. It is not known how P-cadherin and SFK activation impact cell-cell biomechanical properties. Using atomic force microscopy (AFM) images, cell stiffness and cell-cell adhesion measurements, and undirected graph analysis, we demonstrate that P-cadherin overexpression promotes significant alterations in cell's morphology, by decreasing cellular height and increasing its area. It also affects biomechanical properties, by decreasing cell adhesion and stiffness. Cell network analysis showed alterations in intercellular organization, which is associated with cell adhesion dysfunction, destabilization of an E-cadherin/p120ctn membrane complex and increased cell invasion. Remarkably, inhibition of SFK signalling, using dasatinib, reverted these pathogenic P-cadherin induced effects. [Ribeiro *et al.* (2016) *Nanoscale* **8**, 19390–401]

P-1075 (O-209)

Cancer risk and the tree of somatic cell divisionsI. Derenyi¹, G. J. Szollosi^{1,2}

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All cells of an individual are the result of cell divisions organized into a single binary tree. This somatic cell tree is not uniform: some branches are shorter, while others can become much longer. As cell divisions are accompanied by replication errors, longer cell lineages are more prone to the accumulation of mutations and to carcinogenesis. By mapping the accumulation of driver mutations along a somatic cell tree into a graph theoretical problem, we derive a mathematical expression for the probability of developing cancer in an arbitrary cell tree with a given mutation rate. The result is consistent with epidemiological data and highlights the significance of the longest cell lineages. We also show how tissues can minimize the length of their longest lineages through differentiation hierarchies.

P-1077

Use of complementary molecular modeling approaches in search of peptides binding to oncogenic RasL. Buimăgă-Iarincă¹, C. G. Floare¹, A. Farcaș^{1,2}, S. Porav^{1,2}, L. János¹

¹National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania; ²Babeș-Bolyai University, Cluj-Napoca, Romania

Ras proteins mediate a wide signal transduction pathway that regulates cell growth, proliferation and differentiation. Their mutations are responsible for over 85% of specific cancers, and occur in 15–30% of all cancers. Although several approaches were identified, little success in Ras-caused cancer therapeutics was attained. Here we propose an alternative computer-based approach to find peptides that would disrupt oncogenic Ras association.

We first performed molecular docking analysis to find possible specific interactions of peptides to Ras. In this regard we selected over 400 peptides from PepBank with sizes between five and fifteen amino-acids. Evaluating parameters such as: (i) peptide-protein complex geometry, (ii) binding strength and (iii) peptide binding site, we extracted a few peptides that bind to three specific sites.

By means of molecular dynamics simulations we sampled the preferred G12V oncogenic N-Ras self-association binding sites. Combining the two approaches we identified a few highly specific peptides that bind to the aforementioned NRas-NRas binding sites.

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Posters

– 35. Physics of cancer –

P-1078

Study on electric properties measurements of biological tissues by using a microprobe

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Object: Electric properties (EPs) of biological tissues have close connection to its physiological and pathological conditions. In comparison with normal tissues, significant changes of EPs in malignant tissues have been reported. And EPs changes occur before structural changes of biological tissues. It is important to establish a EPs database of cancer tissues and normal tissues for tumor early detection. **Method:** This study designed a small size insertion-type probe for conductivity and permittivity measurements of biological tissues. In order to investigate the suitability of *in-vivo* measurement with the insertion-type microprobe, this study measured EPs of bovine muscle tissues (across and along) and fat tissues in the frequency range from 0.5 to 5MHz. **Results:** It was shown that the EPs values of bovine fat tissues and muscle tissues agreed well with those published data. The data measured by the probe we designed were in good accordance with the simulation results. **Conclusion:** *in-vivo* measurement of biological tissues by using the insertion-type microprobe are feasible. This provide a good experimental method for future study of electric properties measuring of mice tumor.

P-1080

Influence of G12V mutation on NRas proteins' aggregationA. Farcaș^{1,2}, L. Buimăgă-Iarinca², C. G. Floare², L. János²

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Ras proteins are GTPases that regulate signal transduction processes, and are present in three isomeric forms. HRas, KRas, and NRas mutations are detected in about in 15% to 25% of all human tumors, and up to 90% in specific types of cancer. G12V mutants yield permanently activated Ras oncoproteins, resulting in abnormal, uncontrolled growth of cells. Both experimental and computer simulations have shown that membrane-bound Ras proteins form non-overlapping nanosized subdomains (nanoclusters). Dynamics of nanoclusters formation, the nature of protein nanoclusters subcellular localization at different domains and the mechanism of aggregation was investigated for NRas and NRas (G12V). We performed semi-atomistic level molecular dynamics simulations of 36 full-size NRas proteins lipid-anchored in a ternary bilayer model, whose composition is essential for the localization of NRas protein nanoclusters. Contact analysis showed that G12V mutation induces protein-protein interaction loss between residues 134 and 153.

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P-1079

Dynamin related protein 1 alterations regulates migration in melanoma cellsF. dal Yontem^{1,2}, S. H. Kim³, Z. Ding³, S. Ekmekcioglu³, H. Akçakaya¹

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Mitochondria are highly dynamic organelles responding to cellular stress through changes in overall mass, interconnectedness, and sub-cellular localization. Research on mitochondrial fusion and fission gained much attention in recent years, as it is important for our understanding of many biological processes, including the maintenance of mitochondrial functions, apoptosis and ageing. The rate of mitochondrial biosynthesis and degradation can affect various aspects of tumor progression. However, the role of mitochondrial dynamics on melanoma progression remains controversial and requires mechanistic understanding to target the altered metabolism of cancer cells. Therefore, in our study we altered mitochondrial dynamics to evaluate its effect on melanoma progression. Our results show that inhibiting mitochondrial division with mdivi-1 induced cell death and inhibited migration in melanoma cells. However silencing Drp-1 and Mfn 2 induced migration in melanoma cells in contrast with mdivi-1 treatment. We further show that mitochondrial fusion plays a significant role in mitochondrial respiration in melanoma cells. Our findings suggest that targeting molecular mechanisms that regulates mitochondrial dynamics can improve therapeutic approaches in melanoma patients.

P-1081

In silico study of Ras-binding peptides' self-associationL. János¹, C. G. Floare¹, A. Farcaș^{1,2}, L. Buimăgă-Iarinca¹

¹National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania; ²Babeș-Bolyai University, Cluj-Napoca, Romania

Ras proteins self-association (i.e. clustering) is essential in signal transduction of a wide variety of pathways regulating cell growth, proliferation and differentiation. Hence, mutations of Ras are related to over 85% of specific cancers, and to 15-30% of all cancers.

In this work we studied the importance of preliminary peptide self-association on the aggregation of Ras proteins. First, we identified the most important Ras-Ras binding sites, using molecular dynamics (MD) simulations. Then, we selected high affinity specific peptides (from PepBank) binding to these sites by means of molecular docking. Third, we used MD simulations to follow the dynamics and self-association of these peptides in solution (prior to binding to Ras). Fourth, we used MD simulations on systems which include both Ras and the proposed peptides to investigate the effects of peptides on Ras dynamics and aggregation. Finally, we used non-equilibrium free-energy calculations to accurately quantify peptide-Ras binding affinities.

This sum of approaches converge the results into a reliable output describing a complex phenomenon such as the influence of peptides' association on binding to Ras.

We would like to acknowledge the financial support from UEFISCDI grant PN-II-RU-TE-2014-4-2418, contract nr. 118/2015.

Posters

– 35. Physics of cancer –

P-1082

Effects of temperature and substrate on breast cancer cell lines

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In this work the mechanical properties of three breast cell lines were studied in several environments to explore the link between the mechanical properties of cancer cells and their metastatic potential (MP). Using an atomic force microscope, the mechanical properties of MCF-10A, MCF-7 and MDA-231 cells were measured. The measurements were repeated at room temperature and physiological temperature as well as in a standard polystyrene petri dish and petri dishes coated in collagen I and laminin. To expand on previous studies the force curves generated were analysed using three different mechanical models which describe both the elastic and viscoelastic properties of the cells. It was found that in all situations the lower MP MCF-7 cells were more pliant than the high MP MDA-231 cells. The non-cancerous MCF-10A cells were significantly stiffer than the cancer cell lines. By contrasting the mechanical properties of three cell lines of similar origin in the various situations it was found that there is not a predictable relationship between the situation of a cell and its mechanical properties. This builds on previous studies which showed that disparate cell lines react differently to changes in their environment.

P-1084

Estimation of adult patient doses for Chest X-ray diagnostic examinations in a Tertiary Institution

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University of Benin, Benin City, Nigeria

The objective of this study is to use the CALDOSE_X 5.0 software to assess the entrance skin doses (ESD) and effective doses (ED) of adult patients undergoing x-ray examination of the thorax/chest (PA/RLAT), in tertiary institution health centre equipped with constant potential generators (no ripple), an x-ray emission angle of 17° and a total filtration of 2.5 mm Al. In all, 531 newly admitted undergraduate students were surveyed from January to March, 2016. The patients' data and exposure parameters captured into the software included age, sex, examination type, projection posture, tube potential and current-time product. The mean ESD and ED were calculated using the software and compared with published works and internationally established diagnostic reference levels. The mean ESD calculated is 0.29 mGy and the mean effective doses is 0.04 mSv. The values of ESD and ED obtained are below the internationally established diagnostic reference levels, which could be attributed to good radiographic techniques employed during the chest X-ray procedure for these students.

P-1083

On biophysical fundamentals of combined oncotherapy

E. Neu¹, U. Welscher¹, M. C. Michailov¹, J. Foltinova^{1,2}, V. Foltin^{1,2}, R. Stainov^{1,3}, R. Wilkowski¹, N. Willich^{1,4}
¹Inst. Umweltmedizin c/o ICSD/IAS e.V. POB 340316, 80100 München (Berlin-NewDelhi-Paris-Sofia-Vienna, u.a.), Germany; ²Med. Fac., Univ. Bratislava, Slovakia; ³Univ. Fulda, Germany; ⁴Med. Fak., Univ. Muenster, Germany

Introduction & Method: Conservative oncological treatment implies today single and/or combined chemo-(CT: cytostatics), radio- (RT: X-rays), thermotherapy (HT: hyperthermia). Participation of vasomotor reactions is given [Neu, Welscher et al IUPAB-2014 Melbourne, Proc. p.17,31,73-75, -2011 Beijing, Protein&Cell, S19-03, p.113-4,185-6,581-2. J Physiol Sci 59, 241/2009. Acta Physiol 191/658, 52/2007]. **Results** (recent/earlier, n=210): CT: adriablastin, bleomycin, colchicine 0.1-50x10⁻⁶g/ml [A], RT 0.1-50Gy [B] induce contractions of blood vessels (human renal, ovarian, uterine; rat aorta), HT 37-45°C relaxation. Radiogenic contr (RC) are potentiated by Ca⁺⁺, hormones (adrenaline, prostaglandins) [C] or abolished-45°C [D]. Drugs: theophylline, nifedipine, indomethacin [E] antagonize RC. X-rays inhibit EDRF [F]. **Conclusion:** Receptor or store-operated Ca-stretch-channels (Ca-entry by endothelin-1), pericytes could participate in CT/RT/HT. Biophysical analysis of molecular mechanisms (A-F) could help for *modulation of vasomotor reactions via antagonizing of vasoconstriction during CT/RT diminishing their efficacy* (decrease of local substance concentration resp oxygenation) or *of vasodilation* (μ-wave irradiation by hyperthermic effect decrease (heat shock proteins), ie cooling by increased blood flow.

P-1085

Anticancer activity of flavokawain B and its biotransformation product in colon adenocarcinoma cells

A. Palko-Labuz¹, E. Kostrzewa-Susłow², T. Janeczko², A. Pola¹, U. Uryga¹, K. Środa-Pomianek¹, K. Michalak¹
¹Department of Biophysics, Wrocław Medical University, Poland; ²Department of Chemistry, Wrocław University of Environmental and Life Sciences, Poland

Chalcones (1,3-diphenyl-2-propen-1-ones) belong to the family of flavonoid, a large group of plant secondary metabolites. Naturally occurring chalcones and their synthetic derivatives are involved in a wide spectrum of biological activities including anti-inflammatory, anti-invasive, antibacterial, and anticancer activities. We focused on flavokawain B (2'-hydroxy-4',6'-dimethoxychalcone), a component of kava plant (*Piper methysticum*). In our studies, flavokawain B was obtained by chemical synthesis via Claisen-Schmidt condensation. We applied sulforhodamine B assay to determine the growth inhibition of colon cancer cells and it was found that this chalcone has strong cytotoxic activity against colon adenocarcinoma cell line LoVo and their resistant to doxorubicin LoVo/Dx subline. High performance liquid chromatography analysis has demonstrated that in cancer cells flavokawain B can undergo conversion to 5,7-dimethoxyflavanone. We observed considerably lower anticancer activity of the product of this biotransformation as compared with the primary compound. Also, some molecular descriptors of studied compounds were obtained by application of density functional theory DFT/B3LYP/6-31G* using Spartan '16 software and structure-activity relationship was discussed.

Posters**– 35. Physics of cancer –****P-1086****Comparative cytoskeleton and elasticity investigation on healthy and cancerous oral mucosa**M. Strugacevac¹, S. Steeger¹, J. Kristin², M. Glaas², J. Schipper², M. Getzlaff¹¹Institute of Applied Physics, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany; ²Hals-Nasen-Ohrenklinik, Universitätsklinikum Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany

Our group's research deals with hardness and elasticity of carcinoma cells in comparison to benign cells. In order to investigate differences between cell lines, two different techniques are used, fluorescence microscopy and Atomic Force Microscopy (AFM).

Different cell lines from head and neck area were examined by confocal fluorescence microscopy to obtain more information about physical properties of cells. Different markers allow us to highlight specific three main kinds of cytoskeleton filaments: microfilaments, microtubules, and intermediate filaments. Our measurements showed that in tumor cells actin filaments are accumulated in cortical layer what is not noticed by benign cells. Additional differences in cytoskeleton filaments of different cell lines will be shown in this contribution.

Further investigation was focused on mechano-elastic cell properties. AFM was used to examine the differences between cell lines. The Young's Modulus calculated by the Hertzian Model was determined in order to quantify the elasticity of the cells. Latest results concerning mechano-elastic properties of cells will be presented in this contribution as well.

P-1088**Bionanomechanical properties of bladder cancer cells studied with single cell force spectroscopy**

J. Zemla, J. Pabijan, S. Seweryn, M. Lekka

Institute of Nuclear Physics Polish Academy of Sciences, PL-31342 Krakow, Poland

Cancer cells ability to metastasize correlates with both cell and substrate bionanomechanical properties. Here, we report on elasticity and adhesion of bladder cancer cell lines: HCV29, human ureter epithelium, and HTB9, human bladder carcinoma. Single-cell force spectroscopy is used to quantify the contribution of cell adhesion to substrates coated with collagen IV, laminin and vitronectin. The results show that biomacromolecular substrate composition influences on the relative Young's moduli and adhesion forces in a cell-type dependent manner. Biomechanical results correlated with fluorescence microscopy of cell cytoskeleton and focal adhesion organizations.

This work was supported by National Science Centre (Poland) project no UMO-2014/15/B/ST4/04737. The JPK AFM purchase has been realized under the project co-funded by the Małopolska Regional Operational Program, Measure 5.1 – “Krakow Metropolitan Area as an important hub of the European Research Area” for 2007-2013.

P-1087**Darwinian evolution in cancer**

B. Waclaw

University of Edinburgh, UK

Mathematical modelling of cancer has a long history but it traditionally focused on replicating growth laws observed for different tumours, the role of angiogenesis, or predicting the outcome of chemotherapy. Recently, advances in genomics have made it possible to investigate Darwinian evolution in populations of cancer cells. This has opened up many interesting questions. In particular, as the cancerous tumour grows, cells accumulate further mutations. Are these mutations neutral “passengers” (i.e. they do not change the net growth rate) or are some of them “driver mutations” that increase the growth rate? Is there evidence of selection acting on certain traits of cancer cells? How genetically diverse a typical tumour is? How is evolution affected by the spatial structure of the tumour?

In this talk I will show how a statistical physics model can be used to shed light on these problems. I will discuss a 3d lattice model similar to the Eden model which includes four biological processes that are known to be relevant for cancer: replication, death, mutation, and migration of cancer cells. I will show how the predictions of this spatial model compare to previous studies that used well-mixed models. I will then show how the model can be compared to experimental data in order to determine whether new mutations that occur during cancer progression are neutral or not.

P-1089**Short inverted repeats influence localised mutability in human somatic cells**

X. Zou, S. Morganella, D. Glodzki, H. Davies, Y. Li, M. R. Stratton, S. Nik-Zainal

Wellcome Trust Sanger Institute, UK

Selected repetitive sequences termed short inverted repeats (SIRs) have the propensity to form secondary DNA structures called hairpins. SIRs comprise palindromic arm sequences separated by short spacer sequences that form the hairpin stem and loop respectively. Here, we show that SIRs contribute to an increase in localised mutability. Mutability of SIRs is domain-dependent with the greatest mutability observed within spacer sequences (~1.35 fold above background). It is further influenced by factors that increase the likelihood of formation of the hairpin such as loop lengths (of 4-5bp) and stem lengths (of 7-15bp). Increased mutability appears to be an intrinsic property of SIRs as evidenced by the observation that all mutational processes demonstrate a higher rate of mutagenesis of spacer sequences, though it is more marked for tumours that have a high level of APOBEC-related mutagenesis and mismatch repair deficiency. Some SIRs are more mutagenic than others. We identified 88 spacer sequences that show enrichment of between 1.8 fold to 90 fold of local mutability distributed across 283 sites in the genome. These sites can be used to inform the biological status of a tumour.

Posters– *Supplementary poster* –**P-1090****ARBRE-MOBIEU: networking the European molecular-scale biophysical characterization community**

P. England

ARBRE-MOBIEU European Network, France

Molecular-scale biophysics bridges the gap between atomic-scale structural approaches and cellular-level ones. Before 2014, the European core facilities, research infrastructures and shared resource labs in this field (unlike those in other fields such as structural biology or bioimaging) were either isolated or scattered among different organizations, with little opportunity to synergize efficiently. The ARBRE initiative (Association of Resources for Biophysical Research in Europe) was launched to fill this void and to ally the forces in the field of molecular-scale biophysics, as well as to provide it with a clearer identity. More than 100 labs from 25 countries in Europe joined informally and, to cement the group around concrete objectives, decided to put together a successful COST application, called MOBIEU (Molecular Biophysics in Europe) "Between Atom and Cell: Integrating Molecular Biophysics Approaches for Biology and Healthcare". ARBRE-MOBIEU was thus officially launched in April 2016. This poster will summarize the steps taken towards the construction of ARBRE-MOBIEU, and discuss its short- and mid-term networking action plan and strategy.

Website: www.arbre-mobie.eu

P-1091**Multiple structures from one crystal serial crystallography (MSOX): variable temperature movies**S. Horrell¹, D. Kekilli¹, K. Sen^{1,2}, C. W. Yong², T. W. Keal², M. A. Hough¹, R. W. Strange¹

¹School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ, UK; ²Scientific Computing Department, STFC Daresbury Laboratory, Warrington, Cheshire, WA4 4AD, UK

We have shown how multiple structures from one crystal (MSOX) can be used to explore reactions *in-crystallo* and to create movies of an enzyme's entire catalytic cycle [1]. MSOX of Cu nitrite reductase crystals at temperatures from 190–280K and at RT has allowed us to follow, at atomic resolution, the conversion of NO₂[−] to NO and return to the enzyme's water bound resting state, and scrutinise the mechanism proposed by Fukuda [PNAS, 113 2928, 2016] based on a "damage free" XFEL structure. By pairing this structural data with DFT simulations we have demonstrated a clear link between the binding geometry of the NO₂[−] ligand and the redox state of the catalytic type-2 Cu. Catalysis occurs more rapidly with accumulated dose at higher temperatures. At RT, we observed the conversion of NO₂[−] to *side-on* bound NO at the T2Cu, resolving a long standing debate. These results allow us to suggest a multi-temperature approach to gain maximum biological information from MSOX structural movies of enzyme catalysis [2].

[1] Horrell S et al (2016). *IUCrJ* 3 271–281

[2] Sen K et al (2017) *IUCrJ* (submitted)